The American Journal of Medicine

Symposium on Tuberculosis

J. Burns Amberson, M.D.
Guest Editor

November 1950

The American Journal of Medicine

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VOL. IX NOVEMBER, 1950 NO. 5

Symposium on Tuberculosis

Although the use of antibiotics in tuberculosis has thus far fallen short of the dramatic effects achieved in many other infections, their introduction has already met with limited success and has made necessary some reorientation of the principles of management of this disease. The time seems ripe for exposition of these new points of view. Dr. Amberson has enlisted the help of foremost authorities in the field to review the whole subject, beginning with current concepts of the basic aspects of tuberculous infection. The contributors have worked zealously in the presentation of their special interests and the symposium as a whole is a noteworthy integration of present thinking in the field of tuberculosis.

Contents continued on page 5

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*Gray, A. L.: Southern Med. J., 43:320, April, 1950.

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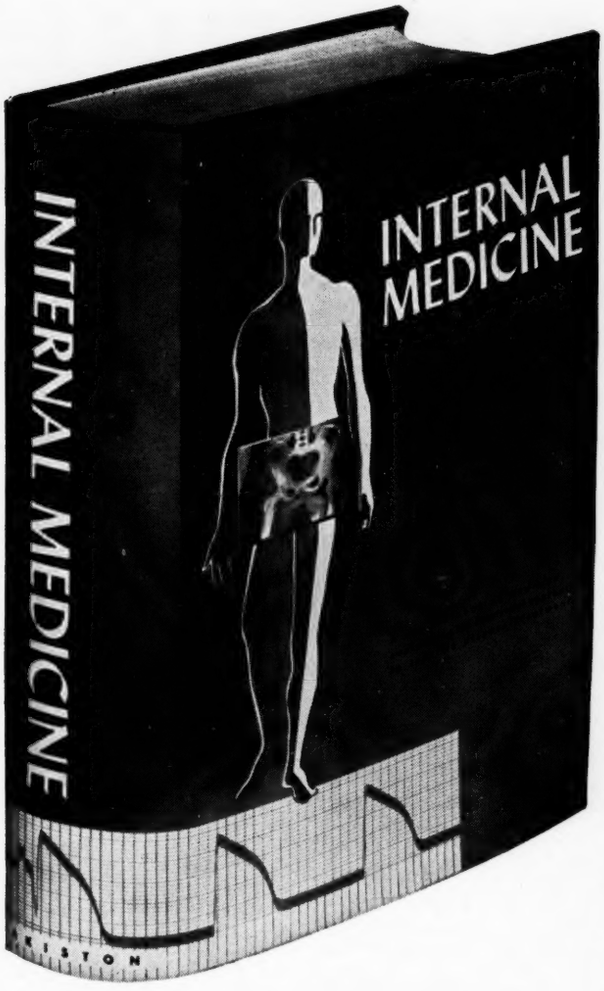

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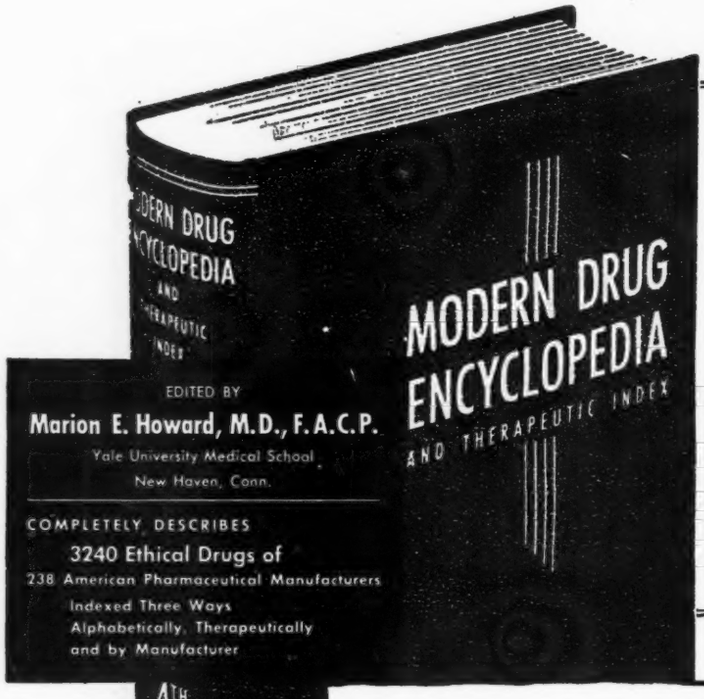

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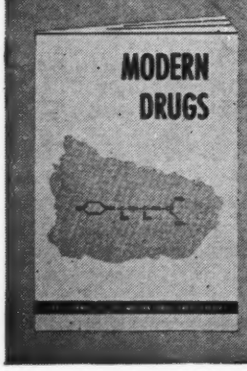

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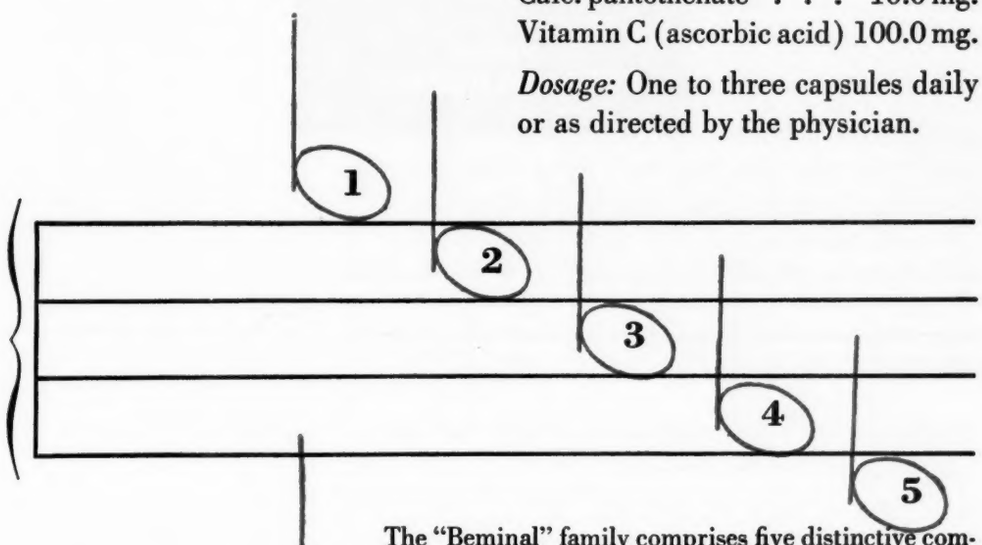

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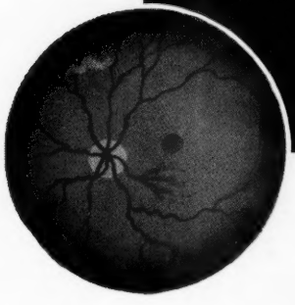
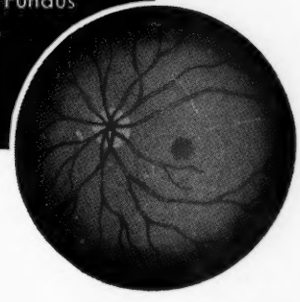


Normal Ocular Fundus



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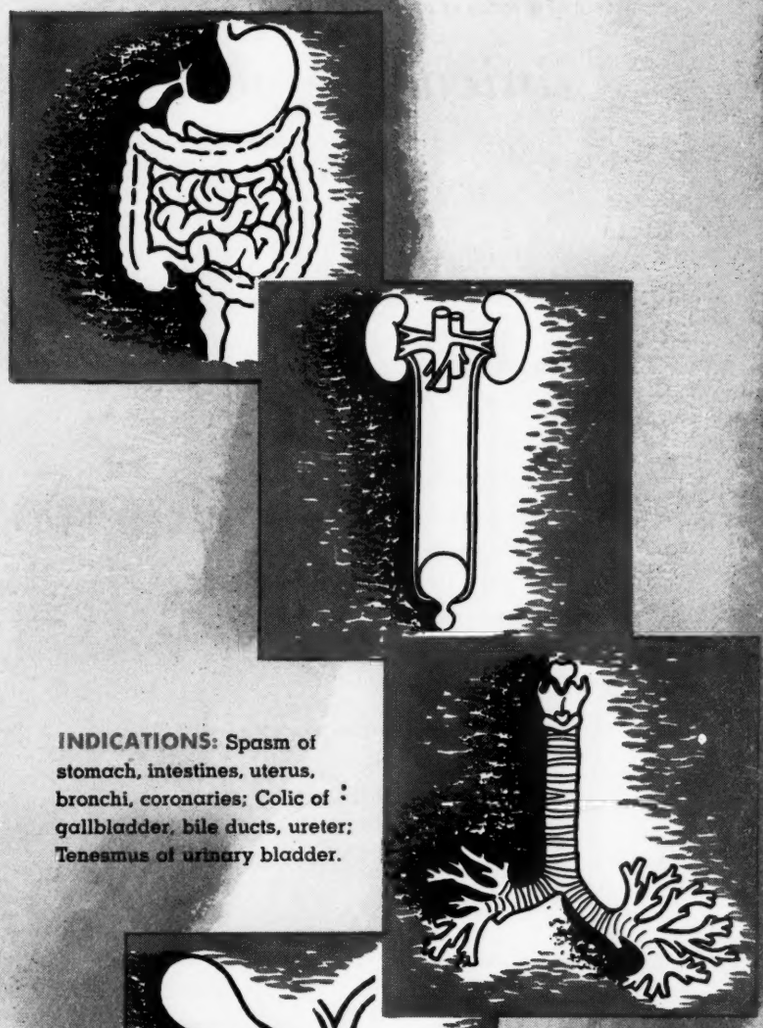

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REFERENCES:

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5. Stephens, G. K.: J. Okla. State Med. Assn., 42:246, 1949.

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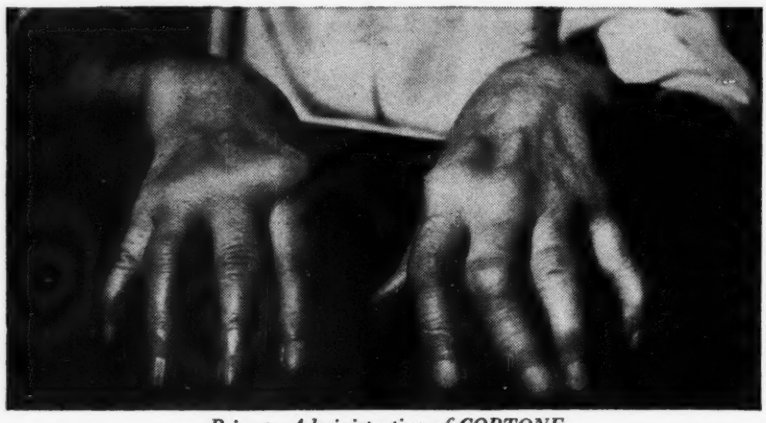

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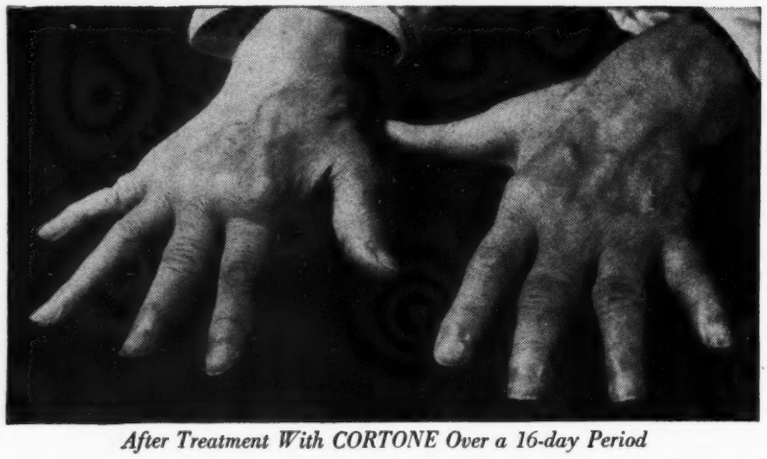

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1. Walker, W.J.: Obesity as a Problem in Preventive Medicine, U.S. Armed Forces M.J. 1:393, 1950.

2. John, H.J.: Dietary Invalidism, Ann. Int. Med. 32:595, 1950.

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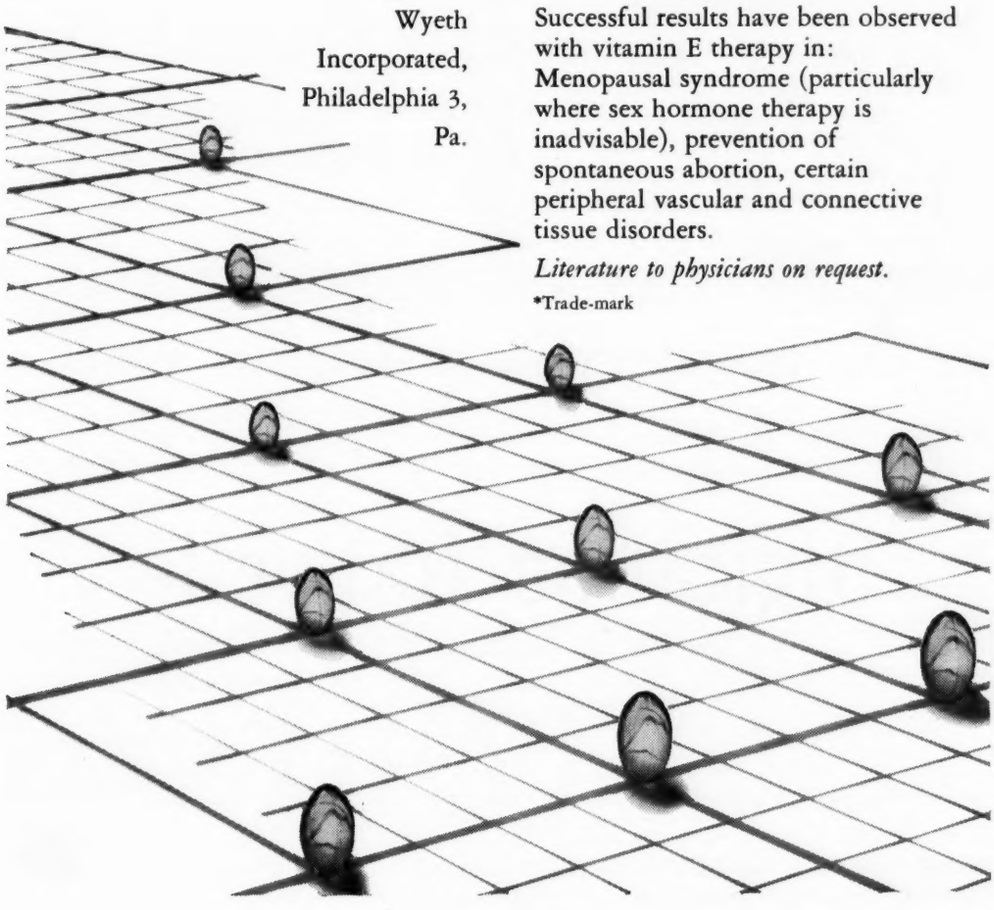

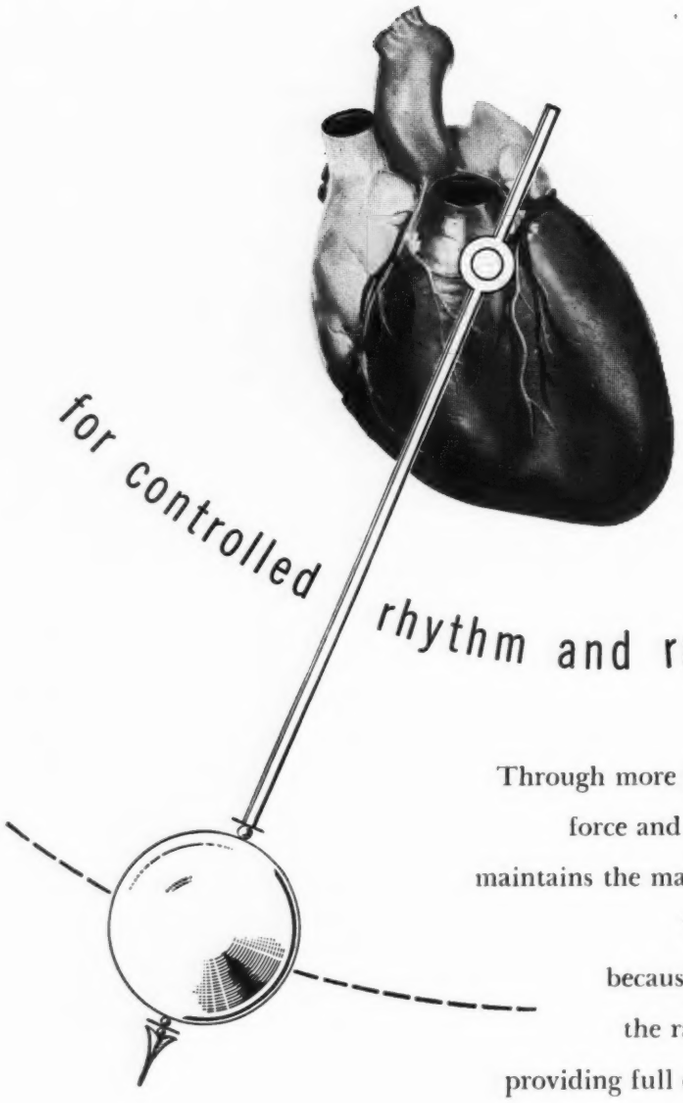

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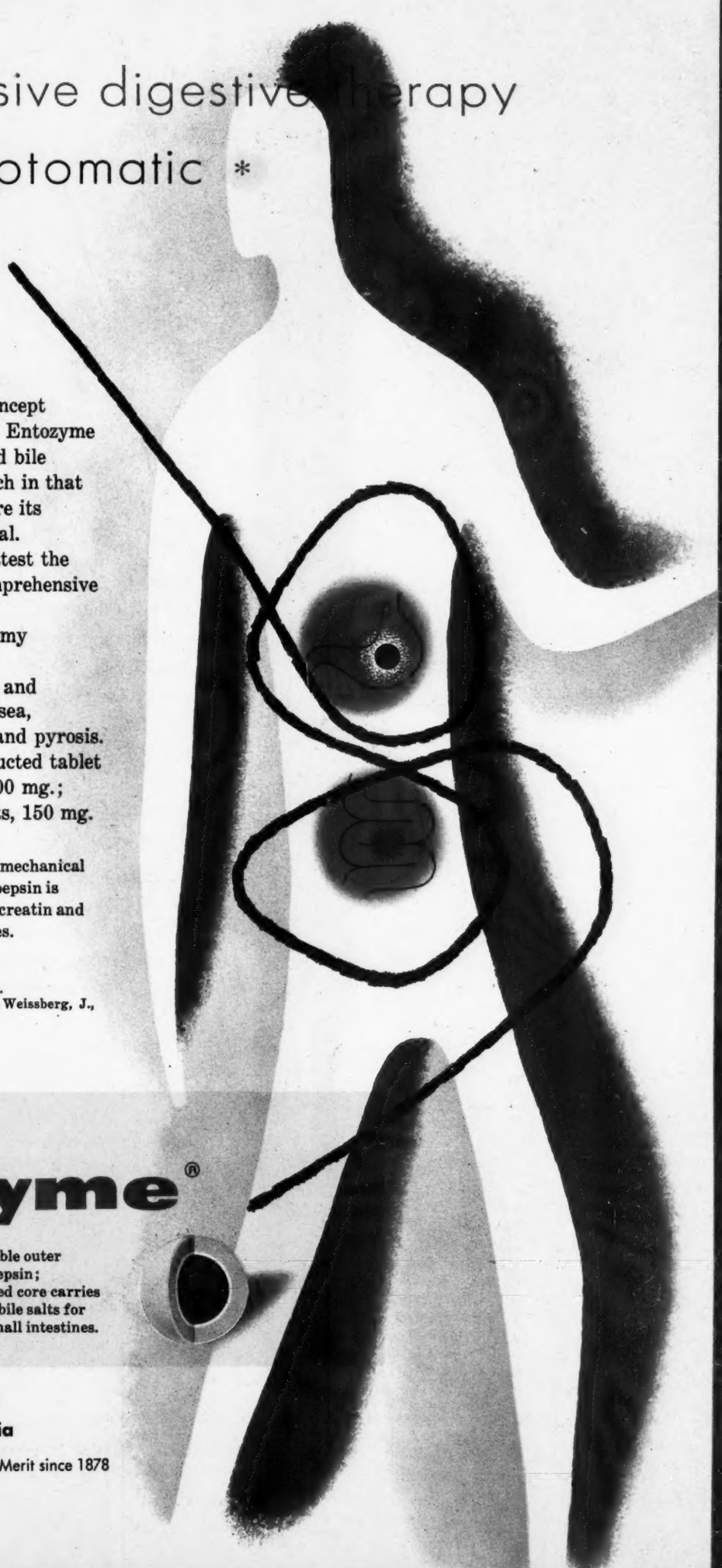

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* A coined word to describe the unique mechanical action of Entozyme Tablet, whereby pepsin is released only in the stomach, and pancreatin and bile salts only in the small intestines.

1. McGavack, T. H., and Klotz, S. D.: Bull. Flower Fifth Ave. Hosp., 9: 61, 1946. 2. Weissberg, J., et al.: Am. J. Digest Dis., 15: 332, 1948.

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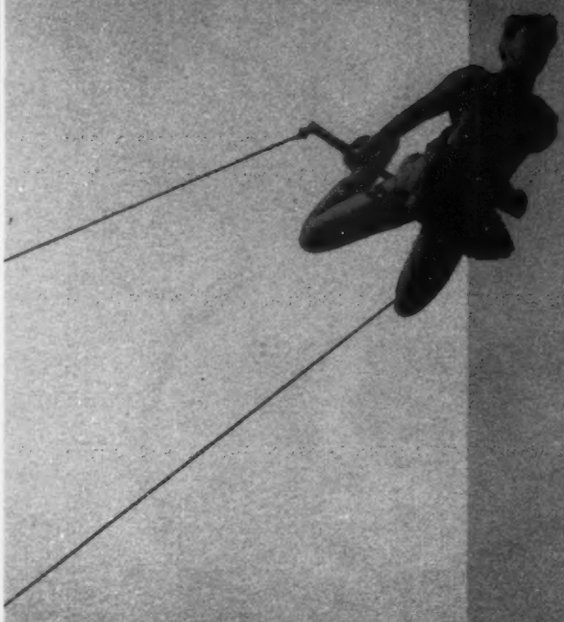

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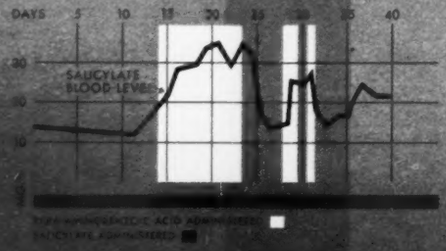
Formula: Each enteric-coated Pabalate Tablet, or each teaspoonful of the chocolate-flavored Pabalate Liquid, contains sodium salicylate, U.S.P. (5 gr.) 0.3 Gm.; para-aminobenzoic acid, as the sodium salt, (5 gr.) 0.3 Gm.



Administration of para-aminobenzoic acid to subjects on a constant intake of sodium salicylate "produced a dramatic rise in the salicylate level in the blood."[2]

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2. Dry, T. J.: Proc. Central Soc. Clin. Research, 19: 69, 1946.
3. Rosenblum, H. and Fraser, [illegible]: Proc. Soc. Exper. Biol. and Med., 65: 178, 1947.

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USES: Acute colds of head and chest, bronchitis, laryngitis, tracheitis, pharyngitis, pertussis, influenza and measles. Helpful as a palliative of harmful cough in tuberculosis, asthma and paranasal sinusitis.

FORMULA: Each 5 cc. (1 teaspoonful) of Robitussin contains:

Glyceryl guaiacolate	100 mg.
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In a palatable aromatic syrup

DOSAGE: Adults: 1 to 2 teaspoonfuls, repeated every 2 to 3 hours as necessary. Children: ½ to 1 teaspoonful according to age, 3 or more times daily.

SUPPLIED: In pints and gallons.

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3. Connell, W. F. et al.: Canadian M.A.J., 42:220, 1940.
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5. Novelli, A. and Tainter, M. L.: J. Pharmacol., 77:324, 1943.

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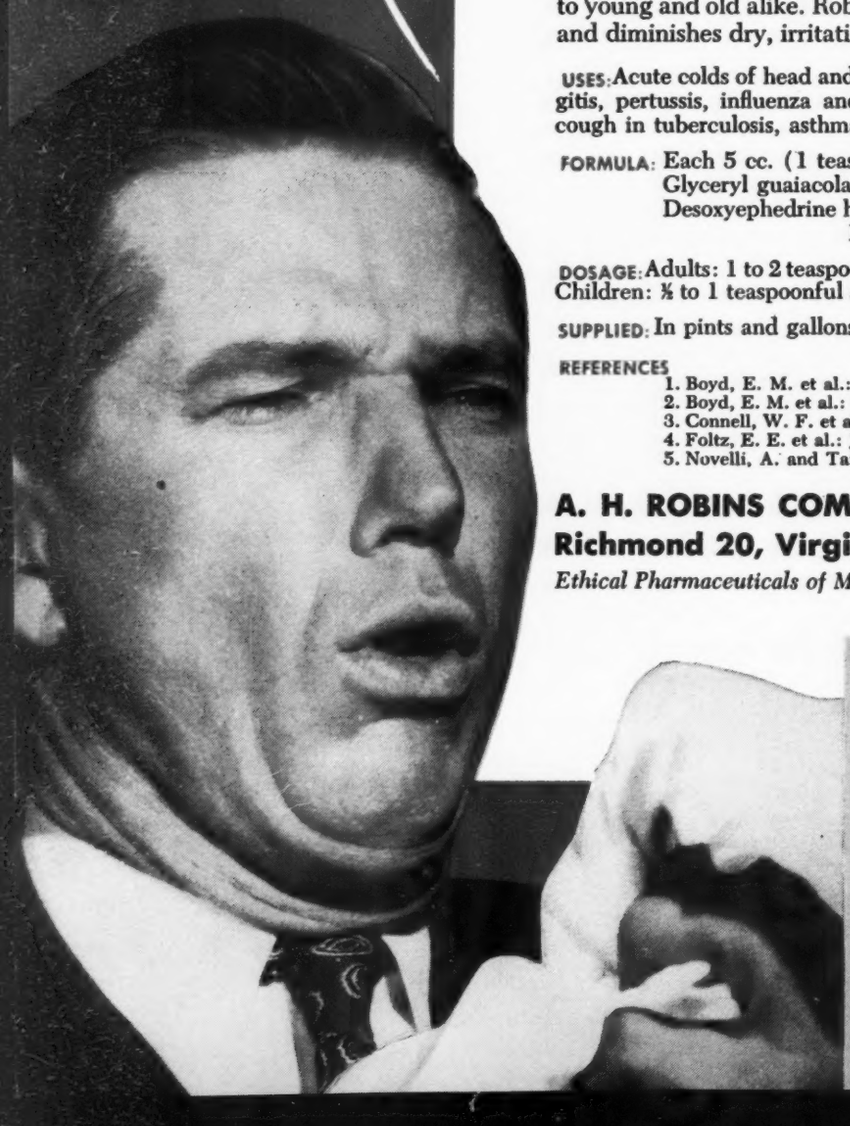

to facilitate productive cough . . .
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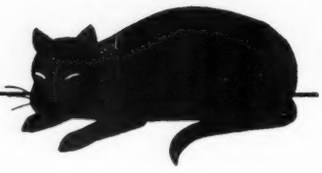

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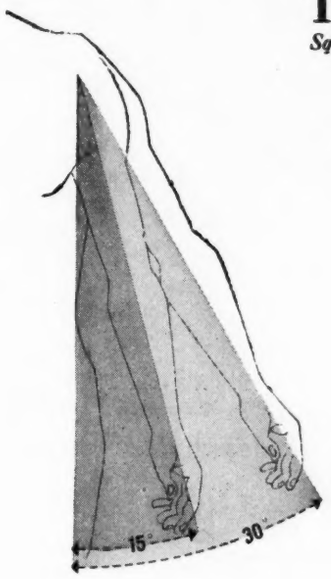
Tolserol

Squibb Mephenesin

- **relieves stiffness and discomfort**
- **permits increased range of motion**



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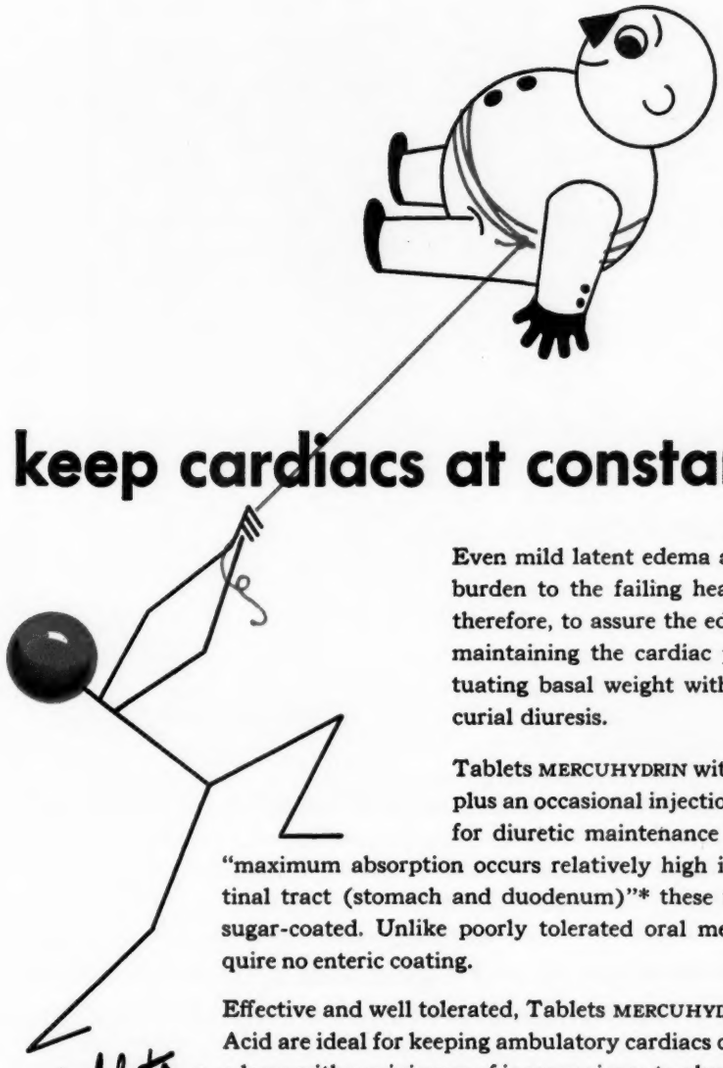

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The American Journal of Medicine

VOL. IX NOVEMBER, 1950 NO. 5

Editorial

Foreword

TUBERCULOSIS probably exerts a more profound influence on the health and longevity of man than any other infectious disease. This is only partly reflected in the estimate that 5,000,000 of the world's people die from it each year among the 50,000,000 afflicted. The figures alone do not reveal many of the ways in which the disease works—its tendency often to strike early in life, to run a chronic course and to cause years of relapsing illness before its fatal ending. The vast social and economic losses reach far beyond the victim. Consequently any plan to control the disease, if it is to be effective, must be predicated not only on scientific medical knowledge but also on recognition of such general influences as working conditions, housing and nutrition. The defenses which are set up are maintained indefinitely since even in countries as favored as the United States, where the death rate is relatively low and steadily falling, the incidence of infection among adults continues to be high.

The most direct and effective preventive of tuberculosis is the removal of all sources of infection. This is demonstrated among cattle in the United States and other countries where systematic elimination of infected members of herds has been accomplished by the simple device of ax and knife. Here now bovine tuberculosis is almost extinct.

The segregation of human beings whose pulmonary cavities are the most common sources of infection among their fellows has its peculiar difficulties and therefore has never been enforced widely. In the first place only a small fraction of those with pulmonary lesions ever become infectious; further, the sloughing and excavation of the lesions in this fraction, a necessary preliminary of infectiousness, is often not attended by outward signs and hence is not detected until many millions of tubercle bacilli have been cast by coughing into the ambient air. Therefore the opportunities for and prevalence of infection always have been great, still are and will continue so for a long time to come. There is urgent practical reason on this account to learn more about the influences which stop a tuberculous lesion near its inception before it undergoes necrosis and sloughing since this usually has the important two-fold effect of preventing progressive disease in the affected individual and avoiding air-borne transmission of bacilli to others. If the disease is not detected near its inception, which is usually the case, a knowledge of these influences is still important because they constitute the basis of medical treatment.

From its earliest beginnings research in the field of tuberculosis has been molded significantly by clinical and epidemiologic observation of its natural behavior. The fact that relatively few of those infected succumb suggests the operation of highly effective native resistance or acquired immunity or both. But the observation that serious deprivations, such as those of great wars, are attended by a prompt upsurge of fatal

tuberculosis and that adverse conditions may precipitate relapses in the individual compels the qualification that these protective mechanisms, whatever their nature, are only relative and not absolute. Relative to what? The varying virulence or fertility of the bacillus? Or is the bacillus constant in its characteristics, the only variables being in the chemical composition of tissues in which it grows? Are bodily defenses genetically predetermined and fixed within limits or do they fluctuate in their effectiveness, and can they by any means be enhanced and kept at a maximum level? These are but a few of the problems which have occupied the thoughts of many generations of students of the disease. With each advance in scientific method the field has been reworked, almost always with the gleaning of new facts to widen our knowledge. At each step the physician has had the obligation to interpret this knowledge—still imperfect—in terms which might benefit his patient. In many respects the patient has indeed benefited materially but still he often suffers because of the imperfections. He, not to speak of the doctor, continues to think wishfully of the impending discovery of a specific cure, ignorant perhaps of the lifetimes spent in this quest and frustrated by the still obscure vagaries of nature which cause our known specific agents to fall short of being curative.

The discovery of a cure, quick, direct and highly effective, would indeed be a boon for mankind. The saving of money would be only minor compared with the elimination of misery and illness now left in the course of the bacillus. But it would be thinking in a most superficial way to look for a "cure" acting similarly, for instance, to penicillin in pneumococcic pneumonia. There are vast differences here which are readily appreciated in the light of knowledge. Longing for the drama of a "cure" may be legitimate, if laid away in the deep recesses of the mind, so long as the working compartments are called upon to guide the many complex and necessary fundamental investigations of the still obscure characters of tuberculosis. Progress in this direction depends upon many of the modern technics of research in a number of scientific fields. It would be profitable to expend large funds to expand the work.

This symposium has been planned to illustrate some of the problems and some of the various approaches to solution. These papers disclose the thinking of serious and highly competent students of tuberculosis who have devoted a major part of their efforts to this subject for many years. They have cooperated generously in preparing the articles and we are most grateful to them.

J. Burns Amberson, M.D.
Chest Service, Columbia University Division, Bellevue Hospital, N. Y.

Symposium on Tuberculosis

Biologic and Immunologic Properties of Tubercle Bacilli*

RENE J. DUBOS, PH.D.
New York, New York

THE true tubercle bacilli constitute only a small group among the many varieties of acid-fast bacilli (mycobacteria) that exist in nature, either in the free-living or in the parasitic state. Their importance comes from their ability to cause in susceptible individuals a progressive and eventually fatal disease that can be initiated by natural infection with small numbers of living cells. It must be recognized, however, that most of the pathologic manifestations of tuberculosis, if not all, can be reproduced by injecting into experimental animals large enough amounts of non-virulent mycobacteria, even of those belonging to the saprophytic free-living species. It is apparent, therefore, that the pathogenic behavior of tubercle bacilli can be analyzed in terms of two independent categories: (1) the factors that endow the bacilli with the ability to proliferate *in vivo* even when only very small numbers of them are introduced into the animal body, and (2) the nature of the bacillary constituents and products that determine in infected tissues the pathologic response characteristic of tuberculosis.

The primary purpose of the present paper is to consider the properties of virulent bacilli which play a part in their pathogenic behavior. Some of the morphologic and physiologic characteristics which are of importance for diagnosis and for cultivation *in vitro* will also be discussed.

CELLULAR MORPHOLOGY

In all types of artificial culture media, as well as in infected tissues, tubercle bacilli occur in the form of rods possessing peculiar staining characteristics. In contrast to other bacteria they bind acid and basic dyes only very slowly at room temperature, but once stained they are extremely resistant to decolorization with acid alcohol. These two peculiar properties have served as a basis for the development of highly selective staining technics (such as the Ziehl-Neelsen technic and staining with fluorescent dyes) but the physicochemical basis of the relation of the bacilli to dyes is poorly understood.

The slowness of the staining reactions is probably caused by the hydrophobic character of the bacillary surface. This hypothesis is supported by the fact that staining can occur rapidly at room temperature when certain wetting agents are added to the dye solution. A modification of the Ziehl-Neelsen technic has recently been described which allows rapid staining at room temperature merely by adding the wetting agent Tween 80 to the solution of carbofuchsin.[5] It is of interest in this respect that Tween 80 is known to be most effective in wetting the surface of tubercle bacilli and in promoting their disperse growth in aqueous media.[15–17]

Among constituents of tubercle bacilli isolated by chemical means only certain hydroxy fatty acids (mycolic acids) have been found resistant to decolorization with acid alcohol after staining with carbofuchsin.[2,4] However, a number of reasons that need not be discussed here make it unlikely that mycolic acid can account entirely for the acid-fast properties of tubercle bacilli. Following the disappear-

* From the Laboratories of The Rockefeller Institute for Medical Research, New York, N. Y.

ance of tubercle bacilli from epithelioid cells the latter are often found to contain fairly large acid-fast globules which probably represent the coalesced remnants of many bacilli, and which consist largely of hydroxy acids (mycolic acid) not readily digested or excreted by tissue cells.[24,38,41]

Although saprophytic mycobacteria are described as acid-fast, most of them exhibit, in reality, only little resistance to decolorization with acid alcohol; they should more properly be called alcohol-resistant rather than acid-resistant. It is worth noting also that certain non-virulent mutant forms of the true tubercle bacilli (described below as Ra variants) are less resistant to decolorization with acid alcohol than are the virulent parent strains. Finally, there seems to be little doubt that in the very early stages of their growth even the virulent tubercle bacilli can be decolorized fairly readily, a fact which may render difficult their detection in the early lesion.[24,38,39,41]

For more than half a century there have been varied and repeated claims that tubercle bacilli may occur in many forms other than the classical acid-fast rods. We have just mentioned that very young cultures often contain non-acid-fast rods and these also occur in old autolyzing cultures. In addition mycelial, granular, amorphous (symplasmic) and even filterable forms of tubercle bacilli have been described in ordinary lesions, cold abscesses, tissue cultures and in cultures in bacteriologic media.[38] But despite many claims no convincing evidence has ever been adduced that materials entirely free of acid-fast rods can give rise to typical cultures of tubercle bacilli or produce progressive tuberculosis. The matter, therefore, must remain *sub judice*.* Yet the recent demonstration in other bacterial species that reproduction can occur by processes other than binary fission, and that hereditary changes of properties can result from sexual-like recombinations, makes it plausible that the classical acid-fast rod may not be the only morphologic expression of tubercle bacilli. The problem presents many experimental difficulties but it bids fair to yield results of great significance in pathogenesis and epidemiology, if the existence of non-bacillary forms of tubercle bacilli is ever proven.

GROWTH REQUIREMENTS

In contrast to the saprophytic mycobacteria, which can grow over a wide range of temperature, the tubercle bacilli multiply at a significant rate only between 35°C. and 41°C. One known exception to this rule exists which provides a striking example of the adaption processes involved in parasitism. A few years ago there were isolated in Australia from ulcers on the extremities of human beings acid-fast rods possessing properties identical with those of mammalian tubercle bacilli except for the fact that they failed to grow at temperatures higher than 34°C.[30] This new type of mycobacterium, referred to as the "Bairnsdale bacillus," causes in man and experimental animals progressive lesions involving both cutis and adjacent subcutaneous tissues, and limited to those anatomic parts with temperatures lower than 37°C. In this case, then, the pathogenic behavior of the organism is conditioned by its temperature requirements for growth. It seems worth mentioning also that strains of tubercle bacilli isolated from lupus lesions of the face exhibit only low virulence for guinea pigs. It has been postulated that this attenuation of virulence may be due to the fact that some of the biologic properties of the organisms are altered by prolonged sojourn near to the

* The origin of the difficulty in demonstrating the existence of viable non-bacillary forms comes from the practical limitations of microscopic technics. Although one living bacillus is sufficient to initiate growth in an adequate culture medium or infection in a susceptible animal, it takes one million bacilli (or clumps of bacilli) per cc. of bacterial suspension before microscopic examination reveals more than one organism per 10 oil immersion fields. It is clear, therefore, that even when bacilli appear "very scarce" or even "absent" by microscopic examination they may be present in large numbers in the material under study. And only the most exacting quantitative technics can justify the conclusion that a given pathologic or cultural material which gave rise to typical growth or infection was entirely free of acid-fast bacilli.

body surface and by contact with an environment different from that prevailing in the internal organs.[21]

As far as is known, tubercle bacilli do not possess a glycolytic mechanism and they derive their energy only from aerobic processes.[25,41] It is true that they can multiply at fairly low oxygen tensions; for example, submerged in the depth of liquid media or in the form of colonies several cm. below the surface in shake tubes of soft agar medium. But under these conditions the extent of growth is severely limited by the rate at which oxygen diffuses through the medium. It seems reasonable to assume that the exacting oxygen requirement of the bacilli plays some part in limiting their multiplication in certain organs *in vivo* and particularly within tuberculous lesions.[42] Thus it has been calculated that the cells of a tubercle respiring at a normal rate would use up oxygen at such a rate that the oxygen tension at the center would be practically nil if the tubercle were more than 0.2 to 0.7 mm. in radius.[25] Although nothing is known of the rate at which oxygen diffuses into areas of caseous necrosis, it is likely that they, too, are under semi-anaerobic conditions. It is not surprising, therefore, that the bacilli do not proliferate in the center of closed caseous areas, whereas they often start multiplying at an enormous rate as soon as abundant aeration is re-established, for example, when the lesion opens into a blood vessel or a bronchus. However, it must be emphasized that increased availability of oxygen is only one of the factors that may be responsible for the multiplication of the bacilli following sloughing out of caseous lesions. As will be pointed out later, repenetration of blood constituents into the caseous material helps in neutralizing the acidic reaction of the lesion and in lowering its content in growth-inhibitory substances. These local biochemical changes probably play an important role in deciding the fate of the bacilli locally present.[14,28]

Tubercle bacilli are capable of synthesizing from a few simple mineral salts and organic compounds all the substances that they need for metabolic and structural purposes. Simple synthetic media containing inorganic nitrogen, a dicarboxylic acid and sugar or glycerine as source of energy allow the production of enormous yields of bacteria provided the culture has access to an abundant oxygen supply. But whereas it is a fairly simple task to obtain abundant growth on synthetic media seeded with large inocula, it is much more difficult to initiate growth of minute inocula containing only a few living cells taken either from cultures or from pathologic materials. Two categories of reasons have been offered to account for this difficulty. On the one hand, it is possible—and, indeed, probable—that although tubercle bacilli possess the ability to synthesize from simple organic compounds most of the complex substances that they need for proliferation, some of their synthetic processes are so slow as to prevent the initiation of growth from a few isolated cells. There are some observations supporting this view. Thus whereas the generation time for early growth from small inocula in serum media is of the order of sixteen hours,[57] bacillary proliferation appears to proceed more rapidly *in vivo* or in association with tissue cultures *in vitro*. Moreover, it has been found recently that one can stimulate markedly the rate of growth of tubercle bacilli by adding fresh chick embryo extract to the culture medium.[8] Extracts of several other tissues fail to increase growth rate in a comparable degree, and the growth-promoting factor present in embryo extract is extremely unstable. Although the chemical composition of this factor is still undetermined, its properties and distribution place it outside the range of known vitamins and other accessory growth substances.

There is overwhelming evidence, on the other hand, that the failure of growth of small inocula is often due, not to the lack of some nutritional element, but rather to the presence in the culture medium of growth-inhibitory substances. Contrary to widespread belief, tubercle bacilli are extremely susceptible to the toxic effect of a large variety of organic and inorganic materials,

such as soaps and other surface-active agents, heavy metals, phenols, quinones, etc., which frequently contaminate the glassware and the ingredients used in the preparation of bacteriologic media.*[,12,15,16] Fortunately, certain organic materials are known—sphingomyelin and serum albumin in particular—which are capable of neutralizing the toxic effect of these substances, probably by forming with them more or less stable complexes. Sphingomyelin and serum albumin facilitate the growth of minute inocula of tubercle bacilli in ordinary bacteriologic media chiefly by virtue of their ability to bind fatty acids which exert a toxic effect when present in the form of unbound salts.[13,15,16]

The two types of media which are used in practice for the bacteriologic diagnosis of tuberculosis take advantage of these facts. Thus the egg media probably owe much of their growth-promoting activity to certain lipids and particularly to sphingomyelin which is an important constituent of egg yolk.†[,13,18] Similarly, addition to synthetic media of 10 per cent serum or of ascitic fluid introduces a concentration of albumin sufficient to neutralize the toxic effect of most impurities. Even more reproducible results can be obtained by replacing serum with an equivalent concentration of serum albumin. In practice, the addition of 0.5 per cent albumin (or 10 per cent serum) to simple nutrient solutions permits the preparation of perfectly transparent liquid and agar media which are very effective in promoting the growth of tubercle bacilli from pathologic materials as well as from pure cultures.

* This fact is frequently overlooked because of inadequate experimental technics. Under ordinary methods of cultivation the bacilli grow in the form of clumps or thick pellicles which are not readily disintegrated into individual bacilli. When suspensions which are inadequately dispersed are inoculated into new media, the organisms at the periphery of large clumps are exposed to the toxic influences and are prevented from multiplying or are killed. These same organisms, however, protect those in the center of the clump, which can then initiate new growth even under inimical environmental conditions. When, on the other hand, the bacillary suspensions used for inoculation consist predominantly of isolated cells or of small clumps, it can be shown by the use of quantitative bacteriologic technics that tubercle bacilli are more susceptible to a greater variety of substances than are most other pathogenic agents.

† It has been found empirically advisable to eliminate from egg culture media most of the egg white. This fact is probably related to the presence in egg white of a substance (presumably lysozyme) which exerts a powerful growth-inhibitory effect on tubercle bacilli.

FACTORS AFFECTING DISPERSED GROWTH

The hydrophobic character of their surface renders tubercle bacilli readily emulsifiable in oil phases. By the same token the bacilli are difficultly wettable by aqueous solution under ordinary conditions *in vitro* and, as a consequence, they tend to adhere to one another instead of growing in a homogeneous dispersed manner in ordinary culture media. Thus arise growths consisting of clumps, heaped masses or pellicles, often reaching relatively considerable dimensions and thicknesses. This peculiar manner of growth causes certain difficulties in experimental work by making it necessary to grind the bacterial mass in order to obtain cell suspensions fine enough for bacteriologic operations. Even more objectionable, probably, is the fact that the bacilli present in the center of the clumps or heaped masses are living under physicochemical conditions vastly different from those prevailing at the periphery of the growth, a fact which results in the development of an heterogenous bacterial population. It is noteworthy, moreover, that the bacilli do not occur in the form of these large masses in tissues but rather usually as very small clumps or isolated cells. In order to work with bacterial populations fairly homogeneous from the physiologic point of view, and less unlike those present in infected tissues than are those yielded by the usual technics, it is often desirable to use culture media which allow dispersed growth of the bacilli. This can be accomplished to some extent by cultivating the organisms in a solution containing 10 per cent egg yolk.[6] It has also been shown during recent years that certain synthetic wetting agents (for example, a polyoxyethylene derivative of sorbitan oleate, commercially known under the name of Tween 80) are extremely effective in wetting the surface of tubercle bacilli

and in causing them to grow diffusely in nutrient liquid solutions.[15–17] By the use of synthetic media containing 0.5 per cent serum albumin and 0.02 per cent Tween 80 it is possible to obtain, within a week's incubation and with all strains of tubercle bacilli, submerged and fairly disperse growths containing 10^9 viable units per cc. of medium. Cultures obtained by this convenient technic possess the morphologic and biologic properties of tubercle bacilli grown in ordinary media and retain in particular their characteristic level of virulence.

EFFECT OF ORGANIC ACIDS ON TUBERCLE BACILLI

As already mentioned, the growth of tubercle bacilli in culture media is readily inhibited by a great variety of organic substances many of which are normal constituents of animal tissues. Thus addition to otherwise satisfactory asparagine-albumin media of lysozyme, or of 0.1 per cent of certain amino acids—methionine or serine, for example—will completely inhibit the growth of many strains.

Most striking and perhaps of special interest from the point of view of the pathogenesis of tuberculosis is the effect of aliphatic acids on bacterial growth. The sodium salts of all fatty acids so far tested, from the lowest (acetic acid) to the highest members of the series (lignoceric acid), can, under certain conditions, inhibit the growth of small inocula of tubercle bacilli in synthetic media.[14] This inhibitory activity can be appreciably decreased but not completely neutralized by addition of serum albumin to the medium. It is very marked at pH 6.5, the optimum reaction for the growth of tubercle bacilli, and increases or decreases as the culture medium is rendered more or less acidic by addition of HCl or NaOH. As appears in Table I, the bacteriostatic activity varies with the length of the carbon chain of the fatty acid, reaching a maximum with capric acid. Interestingly enough, lactic acid can also inhibit growth, even in physiologic concentrations. The mechanism of this growth-inhibitory effect is not yet understood but several facts are known which have a bearing on this problem. First, it is known that the inhibitory organic acids do not interrupt the respiratory processes of tubercle bacilli. Indeed, they enhance their oxygen uptake to a remarkable extent, the *stimulation* of respiration being the greater the more acidic the reaction of the medium, precisely where *inhibition* of growth is also the most effective. It has also been found that these substances (short and long aliphatic acids) can serve as sources of carbon and energy for tubercle bacilli if their concentration in the culture medium is below the growth-inhibitory level. Finally, the bacilli can survive for prolonged periods of time in media containing concentrations of the acids far in excess of those sufficient to inhibit growth. (Table II.) It is clear, therefore, that the growth-inhibitory organic acids do not act as protoplasmic poisons for the tubercle bacilli but only disturb their

TABLE I

APPROXIMATE MOLAR CONCENTRATION OF VARIOUS ORGANIC ACIDS REQUIRED FOR COMPLETE INHIBITION OF GROWTH OF VIRULENT (HUMAN AND BOVINE) TUBERCLE BACILLI IN SEMI-SYNTHETIC MEDIUM CONTAINING 0.5 PER CENT OLEIC ACID-ALBUMIN COMPLEX (pH 6.4)*

Acid	Agar Medium		Liquid Medium	
	Human Strain	Bovine Strain	Human Strain	Bovine Strain
Acetic	0.0040 M	0.0050 M	0.0050 M	0.0050 M
Propionic	0.0030 M	0.0080 M	0.0030 M	0.0060 M
Butyric	0.0015 M	0.0030 M	0.0020 M	0.0060 M
n-Caproic	0.0002 M	0.0004 M	0.0003 M	0.0005 M
n-Caprylic	0.0003 M	0.0004 M	0.0002 M	0.0004 M
n-Capric	0.0001 M	0.0002 M	0.0001 M	0.0002 M
Lauric	0.0002 M	0.0002 M	0.0003 M	0.0003 M
Palmitic	0.0005 M	0.0004 M	0.0010 M	0.0010 M
Stearic	0.0008 M	0.0006 M	0.0010 M	0.0010 M
Oleic	0.0010 M	0.0006 M	0.0010 M	0.0010 M
Lactic	0.0080 M	0.0050 M	0.0100 M	0.0040 M
Pyruvic, Ketoglutaric, Glutamic, Succinic, Fumaric	No inhibition at 0.05 M		.....	.....

* Experimental details described in reference[14].

normal metabolic processes and thereby produce a reversible bacteriostatic effect. In contrast to the aliphatic compounds and to lactic acid, the dicarboxylic and keto acids (glutamic, succinic, fumaric, pyruvic, ketoglutaric acids, etc.) enhance growth at all

TABLE II
SURVIVAL OF TUBERCLE BACILLI (BOVINE STRAIN) IN TWEEN-ALBUMIN MEDIA CONTAINING INHIBITORY CONCENTRATIONS OF ORGANIC ACIDS*

Concentration of Organic Acid in Liquid Media	Growth in Liquid Media	Number of Viable Organisms per cc. of Liquid Medium after Incubation at 37°c. for:					
		1 hr.	1 day	2 days	5 days	8 days	13 days
0	+++	10^5	10^5	10^6	10^7	10^8	10^8
Lactic acid 0.5	–	10^5	10^5	10^5	10^5	10^4	10^2
Butyric acid 0.05	–	10^5	10^5	10^5	10^5	10^5	10^4
Capric acid 0.005	–	10^5	10^5	10^5	10^5	10^4	10^3
Oleic acid 0.005	–	10^5	10^5	10^5	10^5	10^4	10^1

* Experimental details described in reference[14].

pH and in all concentrations so far tested (up to 0.4 per cent).

As the growth-inhibitory or growth-stimulatory effects brought about by aliphatic and lactic acids occur at concentrations well within the levels that can exist in living tissues, it does not seem unreasonable to assume that the effects observed *in vitro* may be of some significance for bacillary multiplication *in vivo*. Thus it has long been known that inflammatory areas become acidic (pH 6.5), at least during certain phases of their evolution, and contain organic acids in high concentrations. This may be due to the fact that these areas are the seat of anaerobic metabolism and that mononuclear cells have a powerful glycolytic metabolism.[31] It has been claimed also that caseous material is rich in long-chain fatty acids probably released by lipase action during partial autolysis of necrotic tissues.[55] The presence of these substances and the prevalence of acidic reaction must constitute an unfavorable environment for tubercle bacilli and may play a role in their progressive disappearance from the center of necrotic lesions. One may even wonder whether the particular type of fatty acid released during autolysis of necrotic tissue may not be of importance in this respect, capric acid, for example, being much more inhibitory *in vitro* than stearic, oleic or lignoceric acids, whereas the latter are much more likely to enhance bacterial multiplication if their concentration is not excessive. Compatible with this view is the fact, already mentioned, that the number of tubercle bacilli detectable in the center of the caseous lesion decreases slowly and continuously as long as the lesion remains closed whereas the bacilli often start proliferating again when the caseous material softens by repenetration of blood constituents or when the lesion opens into a bronchus or blood vessel. It is true that, as mentioned earlier, increased availability of oxygen may play a part in the release of bacteriostasis. But it is also possible that the ease of exchanges between the lesion and the body fluids may effect in a profound manner the suitability of the environment for bacillary growth by neutralizing the local acidity and decreasing the concentration of growth inhibitors. In other words, it appears worth while to consider that the fate of the bacteria present in the lesion is determined not only by changes in oxygen tension but also by the local concentration of organic acids and by the efficiency of the acid-base exchanges at the site of the lesion.

Experiments by Lurie are worth recalling in this respect.[28] He inserted into the peritoneal cavity of rabbits silk bags containing virulent tubercle bacilli and impregnated with collodion in such a manner as to permit the passage of body fluids but not of cells. Under these conditions bacterial multiplication proceeded unchecked in the bags placed in normal animals whereas little or no growth developed in those in immunized animals. Lurie observed that the reaction of the fluid in the bags in the immunized animals was more acidic than that of the fluid in normal animals but in no case was the pH low enough (approximately 7.0) to account for bacteriostasis. As phagocytic cells had not penetrated the bags it appeared that bacteriostasis was caused

by some humoral antibody. The results reported in the present paper suggest a different interpretation of Lurie's findings. Lowering of the pH in the immunized allergic animal was probably the result of local accumulation of organic acids caused by the inflammatory reaction, and it seems possible that bacterial growth was inhibited not by the low pH nor by humoral antibodies but as a result of the local accumulation of one or several of the organic acids known to exert a bacteriostatic effect on tubercle bacilli.

In order to prevent misunderstanding it may be necessary to point out here that the presence of organic acids in inflammatory and caseous areas constitutes only one of the several factors which may affect the course of tuberculous infection. Tubercle bacilli are readily phagocytized, and it is probably within the phagocytic cells that the most effective processes of immunity take place. There is, indeed, much evidence that the ability of the infected host to overcome infection ultimately depends upon the power of its monocytes to kill the bacilli that they have engulfed.

DETERMINANTS OF PATHOGENICITY

When cultures of tissues taken from normal animals are inoculated *in vitro* with virulent tubercle bacilli, an apparently symbiotic relationship becomes established between the bacterial and mammalian cells.[10,29,38] The bacilli are rapidly phagocytized but continue to multiply and are soon found in enormous numbers within the phagocytes, which also continue to increase in number. Under these conditions both bacterial and mammalian cells retain a healthy appearance for prolonged periods of time, as if they were indifferent to each other's presence; moreover, the symbiotic relationship between bacilli and phagocytes can be maintained through several subcultures *in vitro* by inoculation into new media. Thus tissue culture studies provide no evidence that virulent tubercle bacilli produce any toxin having an obvious cytotoxic effect on phagocytic cells, or that the latter have any significant inhibitory effect, in the absence of humoral factors, on the multiplication of the bacilli that they have phagocytized. Similar conclusions have been derived from histologic studies of the early response elicited by the injection of virulent tubercle bacilli into the skin of normal animals. For a few days the bacilli multiply freely without calling forth any striking reaction beyond that caused by the trauma of injection; they behave as almost inert intracellular parasites. It is only after ten to fifteen days that the lesion takes on a more characteristic aspect, when intense inflammation begins to become manifest. Even with large infective doses tubercle formation and caseation require a minimum of two weeks before becoming evident.[24,38,41] It seems worth pointing out in this respect that multiplication of the bacilli inside the intact mononuclears without any evidence of injury is not encountered only during the first phase of tuberculous infections. The phenomenon occurs also in Johne's disease of cattle and in lepromatous leprosy of man, two mycobacterial diseases which exhibit little evidence of allergy of the tuberculin type.[27]

The course of the initial tissue reaction is dramatically different when living tubercle bacilli or their breakdown products are injected into tuberculous animals or into animals that have been rendered specifically hypersensitive. In this case the bacillary materials immediately behave as powerful poisons. They elicit at the site of injection an acute exudative response which goes on rapidly to the development of productive tubercles or to caseation, the whole process taking place within two to four days.[24,38,41] The necrotic effect of bacillary products on hypersensitive tissue can be recognized, not only by injecting these products into the whole animal, but also *in vitro* by means of tissue culture experiments.[41] Cultures of cells taken from tuberculous or hypersensitive guinea pigs are either killed or their migration and proliferation inhibited by the addition to the medium of tuberculin in concentrations so small as to be in-

nocuous for the cells taken from normal animals of the same species.

Thus it is fair to assume that several aspects of the pathology of tuberculosis are the expression of the peculiar necrotoxic effect of tuberculin-like substances on tissues rendered hypersensitive to them by prior exposure to tubercle bacilli. Several distinct proteins or peptides of tubercle bacilli appear to possess the ability to elicit the delayed type of allergy known as tuberculin reaction.[7,46] It has been claimed, furthermore, that the different tuberculo-proteins differ qualitatively in the manifestations that they elicit, some being most active in bringing about systemic toxicity and death in allergic animals, others being chiefly effective in eliciting the delayed type of skin allergy.[22,31,56] Unfortunately, far too little is known of the biologic and physicochemical properties exhibited by tuberculoproteins in their native form to allow dogmatic statements concerning these important questions at the present time.

Although hypersensitization caused by prior exposure to the bacilli increases in a dramatic manner the response of tissues to the tuberculo-proteins, it is also true that all the bacillary constituents and products possess biologic activities which are independent of allergic sensitization, and that the injection of killed bacilli into normal animals can cause several of the effects which accompany virulent infection. Cachexia and death within weeks or months can result from the parenteral administration of sufficiently large amounts of bacillary material whereas smaller amounts will produce at the site of injection many of the histopathologic reactions of tuberculosis. During recent years efforts have been made to analyze these phenomena in terms of the cellular response of tissues to each one of the separate constituents of tubercle bacilli obtained in a more or less purified form by chemical fractionation. The findings can be summarized as follows:[24,38,41,44]

The tuberculo-polysaccharides attract the polymorphonuclear leukocytes and cause an immediate outpouring of young neutrophiles from the bone marrow. When the neutrophiles begin to degenerate at the end of the first day, they are phagocytized by macrophages. The first reaction to the tuberculo-proteins is also an accumulation of neutrophiles soon followed by mobilization of monocytes. When insoluble tuberculo-proteins are taken up by monocytes, these rapidly change into epithelioid cells and the process finally results in the formation of giant cells of the Langhans or foreign-body type.

Although the tuberculo-proteins can elicit in tuberculous individuals the delayed type of skin reaction known as tuberculin reaction, they are incapable when injected alone of inducing by themselves the state of tuberculin hypersensitivity in normal animals. It has been recently shown, however, that this can be done by the simultaneous injection of the proteins in admixture with the wax fraction of tubercle bacilli.[40]

The bacillary fraction consisting of the acetone insoluble part of the alcohol-ether soluble lipids is readily ingested by monocytes and causes the latter to be transformed into epithelioid cells. These accumulate in the form of epithelioid tubercles which simulate closely the strictly productive tubercles of the natural disease. During this process epithelioid giant cells are formed by nuclear division not accompanied by cytoplasmic division. Later the epithelioid cells may die singly or *en masse*, the latter process producing a picture somewhat similar to caseation.[44]

Among the pathologic manifestations of tuberculosis one of the most characteristic and important is caseation necrosis. Although the immunologic and biochemical reactions involved in caseation and liquefaction have not been elucidated, it would seem that caseous matter is produced when necrotic tissue fails to undergo the orderly and complete autolysis that would permit its resorption. Histopathologic observations are compatible with the view that certain components of tubercle bacilli, or some products of their activity *in vivo*, are capable of interfering with one or several of the

enzymatic reactions concerned in complete resolution. It has been shown, indeed, that crude fractions of tuberculo-phosphatides and tuberculo-polysaccharides can interfere *in vitro* with certain types of proteolytic reactions but the relation of this finding to the events *in vivo* that result in caseation has not yet been established.

Although many of the phases of tuberculo-histogenesis have thus been caused to occur by the injection of purified bacillary fractions, other aspects of the natural disease have not yet been reproduced in the absence of actual infection with living organisms; for example, this is the case for the exudative response, prolonged fever, loss of weight and toxemia. It is not unlikely, however, that these pathologic and symptomatic manifestations of tuberculosis will eventually be shown to be due to the metabolic disturbances caused by the multiplication of the invasive micro-organisms and, more particularly, by the varied allergic reactions that they elicit in the hypersensitive host.

DETERMINANTS OF VIRULENCE

The facts outlined in the preceding sections illustrate that it is possible to reconstruct many of the phases of tuberculosis in terms of the characteristic response of tissues to the biologic activities of certain bacillary proteins, carbohydrates and lipids. It must be borne in mind, on the other hand, that these tissue responses can be evoked not only by products obtained from virulent strains of tubercle bacilli but also by products separated from strains which are incapable of producing progressive disease, i.e., which have become avirulent or at least attenuated. It is possible that loss of virulence is due merely to the fact that quantitatively these attenuated strains are not quite as effective as their virulent counterparts in eliciting tubercle formation, or tuberculin allergy, or caseation necrosis. On the other hand, the inability to produce progressive disease may be due to differences of an entirely different qualitative order, and may depend upon the fact that the attenuated and avirulent variants have become unable to *multiply in vivo*. There is as yet no adequate information to choose between these two alternatives and it is possible to present only some facts that may be relevant to the problem. These facts have been derived from a comparative study of the morphologic and biochemical characteristics exhibited by the following classes of tubercle bacilli possessing different degrees of virulence: (1) the fully *virulent* forms capable of causing progressive and fatal disease in normal animals of susceptible species; (2) the *attenuated** forms which have lost the ability to cause progressive disease in normal animals although they are still able to give rise to a limited degree of multiplication *in vivo*, producing lesions that heal spontaneously after a few weeks to a few months. Representative of this group are the human strain, R1Rv,[42] which rapidly lost virulence during cultivation on artificial media, and the classical BCG,* an attenuated strain of bovine type widely used for immunization against tuberculosis.[11] Both these strains are still capable of causing caseation and death in silicotic guinea pigs; (3) the *avirulent** variants, also derived from virulent forms but which seem to have become totally incapable to multiplying *in vivo*. Representative of this group are the cultures H37Ra, JH16Ra and R1Ra isolated at the Trudeau Sanatorium.[49]

Striking differences have been recognized between the manner of growth of virulent cultures of mammalian bacilli and that of many of the avirulent variants derived from them.[36] In all fully virulent cultures the bacilli tend to adhere to one another in the direction of their long axis, thus forming long strands giving to the young growth a serpentine pattern which spreads as a veil over the surface of solid or liquid oleic acid-albumin agar. On the other hand, all

* The BCG culture is often referred to as "avirulent." Its properties correspond rather to those of the "attenuated" strains which, according to Pasteur's original definition, are capable of giving rise to a self-limited immunizing infection but not to fatal disease. We prefer to use the adjective "avirulent" for the variant forms which, like H37Ra, appear unable to multiply *in vivo*,

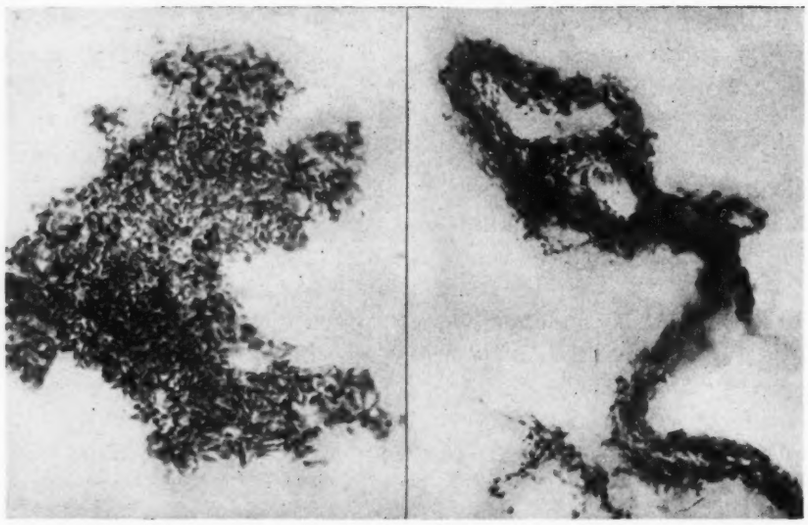

FIG. 1. Left, Ziehl-Neelsen stained preparation of an eight-day old culture of avirulent tubercle bacilli (H37Ra) grown in oleic acid-albumin medium; the bacilli are not oriented and form clumps. × 1520. Right, Ziehl-Neelsen stained preparation of virulent tubercle bacilli (H37Rv) grown in oleic acid-albumin medium; the bacilli form serpentine strands. × 1520. (From DUBOS, R. J. Bacteriological aspects of the pathogenesis of tuberculosis. *Bull. Mt. Sinai Hosp.*, (in press).

cultures tested thus far which grow in an unoriented manner in oleic acid-albumin media have been found to be avirulent. The attenuated strains (BCG in particular) are somewhat intermediate in morphology. (Figs. 1 and 2.) However, it will be emphasized later that these statements are not entirely valid in the case of cultures belonging to the attenuated group since several of them exhibit a serpentine pattern of growth indistinguishable from that of the fully virulent forms.

It appears likely that the serpentine pattern of growth is due to the presence around the bacteria of a hydrophobic lipid causing the individual cells to adhere to one another in the direction of their long axis. This hypothesis has been substantiated by the discovery that the typical strands of virulent tubercle bacilli can be rapidly dispersed into their individual component cells, even at low temperature, by treatment with a variety of hydrocarbons, such as petroleum ether.[9] It is of particular interest that disintegration of the bacillary strands can be achieved without affecting the staining characteristics and the viability of the bacilli, a fact which suggests that the lipid responsible for the serpentine pattern is not an essential part of the bacillary structure but merely exists as an outer layer of the cell.

Little is known of the role played by this lipid in the pathogenesis of tuberculosis. It has been shown recently that the cells, living or dead, of virulent tubercle bacilli can inhibit the migration on coagulated plasma of the phagocytes that have engulfed them whereas the avirulent variants are much less active in modifying the rate and extent of leukocytic migration even though they are as readily phagocytized as their virulent counterparts.[1,32] Moreover, a certain correlation appears to exist between the susceptibility of a given animal species to a given type of tubercle bacilli (mammalian or avian) and the susceptibility of the cells of this species to be inhibited in their migratory activity by the corresponding bacillary type. Thus virulent avian bacilli inhibit the migration of avian (but not of guinea pig) leukocytes whereas virulent mammalian bacilli affect the leukocytes of the guinea pig but not those of the chicken. It is of considerable interest, therefore, that when the lipid fraction released from viru-

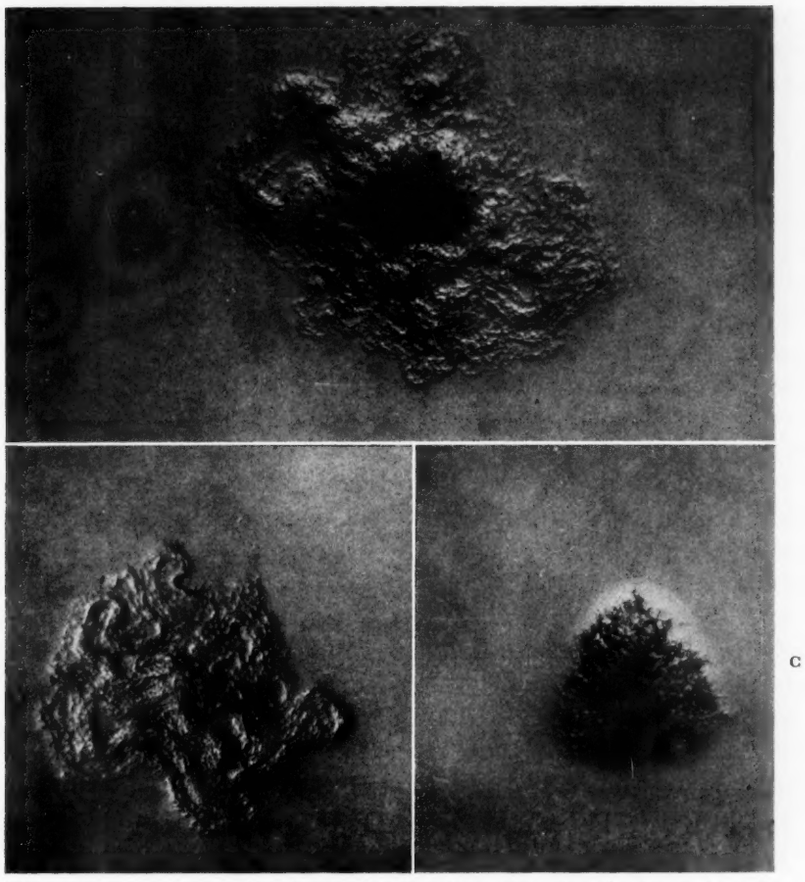

FIG. 2. Comparative colonial morphology of three strains of tubercle bacilli: A, BCG (strain obtained from the Henry Phipps Institute); B, H37Rv (virulent human strain); C, H37Ra (avirulent variant of H37Rv). All colonies are from a nine-day old growth on oleic acid-bovine albumin agar. × 230. (From DUBOS, R. J. Bacteriological aspects of the pathogenesis of tuberculosis. *Bull. Mt. Sinai Hosp.*, (in press).

lent mammalian tubercle bacilli by extraction with petroleum ether is adsorbed on otherwise inert particles, it confers upon them the ability to inhibit the migration of guinea pig leukocytes.[9] Mention should also be made of the fact that repeated injections into mice of small amounts of this crude lipid resuspended in oil brings about vascular pulmonary lesions and death of the animals after a period of nine days or longer. The same fraction, however, appears entirely non-toxic for mice when injected as a single dose, even in large amounts. Because of the fragmentary and preliminary nature of these findings it is difficult to evaluate at the present time their significance in the pathogenesis of tuberculosis, especially in view of the fact that certain saprophytic mycobacteria and even animal tissues also yield lipid fractions with properties similar to those of virulent tubercle bacilli.

The observations reported so far appear to suggest some correlation between the serpentine pattern of growth of tubercle bacilli and their pathogenic activity but it is also true that the virulent and avirulent forms differ in many characteristics other than the morphologic appearance of their growth. A few of these differences may be listed here.

The avirulent variants (H37Ra, JH16Ra and R1Ra) are much less acid-fast than the virulent and attenuated strains. Indeed, these Ra forms are essentially non-acid-fast when growing as submerged colonies in a

soft agar (0.1 per cent) medium containing glucose. Whereas all strains that exhibit the serpentine pattern of growth are able to bind the dye neutral red in the form of its red salt even in strongly alkaline media (sodium barbiturate or 0.1 N NaOH), the Ra forms allow the dye to become yellow under the same conditions. In comparison with the virulent forms from which they are derived the avirulent variants are usually more difficult to cultivate *in vitro* and they appear to have more exacting nutritional requirements. They are more susceptible to the toxic effect of certain organic acids and of Zn^{++} and Mn^{++} ions; but, on the other hand, they are better able than the virulent forms to reduce methylene blue in dehydrogenase experiments of the Thunberg type. Some of these differences may be merely different manifestations of the same fundamental character but it is also possible that a few correspond to independent variations. In any case, it is likely that the loss of the serpentine pattern of growth (and of the ability to produce the particular lipid mentioned earlier) constitutes only one of the many alterations in cellular structure and properties that occur when tubercle bacilli change from the mode of growth typified by the H37Rv (virulent) form to that typified by H37Ra.

Brief mention of some of the properties characterizing the attenuated cultures may help in analyzing the mechanism of pathogenic behavior. The attenuated strain R1Rv, which has become unable to produce progressive disease in guinea pigs, still continues to grow in the form of serpentine strands indistinguishable from those produced by the fully virulent strains. Similarly, it is possible to isolate from the attenuated bovine strain, BCG, substrains which exhibit the serpentine pattern on oleic acid-albumin medium but which, like R1Rv, are unable to cause fatal infection.[50] How are these facts to be reconciled with the hypothesis that the serpentine pattern of growth is the earmark of the virulent forms? Several possibilities come to mind.

One might assume that the various bacillary strains produce different amounts of the lipid responsible for the serpentine strands. According to this hypothesis R1Rv and BCG, for example, would produce less of it than the fully virulent forms but still enough to cause the bacilli to adhere one to the other in the direction of their long axis. This amount would not be sufficient to allow maximum virulence but would permit a certain extent of multiplication *in vivo*.

It is also possible that there exist qualitative differences in the characteristics of the specific lipids produced by the various strains of mycobacteria and that R1Rv and BCG produce substances inefficient in endowing these cultures with virulence.

Finally, one may consider that loss of virulence can result from alterations unrelated to the production of serpentine strands but affecting, by entirely different mechanisms, the ability of the bacilli to multiply or survive *in vivo*. This hypothesis is supported by quantitative bacteriologic studies of the fate of tubercle bacilli of various degrees of virulence injected into normal animals.[26,50] Whereas the avirulent forms (H37Ra) probably do not multiply at all *in vivo*, the attenuated strains (R1Rv and BCG, for example) increase in numbers at the same rate as the fully virulent strains during the first few days of the infection. After a certain length of time, however, the multiplication of R1Rv or BCG stops instead of continuing long enough to produce progressive disease as is normally the case with the virulent strains. These findings indicate that, under the conditions of the experiment, certain processes come into play within one or two weeks after infection which are capable of inhibiting the growth of the attenuated forms but which are much less effective against the fully virulent forms. These growth inhibitory processes have not yet been identified but it is known that they fail to function at the site of lesions produced by silica dust.

Silicosis enhances the susceptibility of man to pulmonary tuberculosis. Similarly, silica dust introduced by subcutaneous

injection or by inhalation into guinea pigs or mice produces local conditions which favor the multiplication, not only of virulent, but also of attenuated tubercle bacilli, rendering the latter capable of causing caseation, cavitation and death.[51] Enhancement of infection by silicosis is not due to an increase in the intrinsic virulence of the infective agents, for the cultures recovered from silicotic lesions exhibit the original level of virulence characteristic of the culture injected into the silicotic animal. For example, bacilli recovered from guinea pigs dying of silico-tuberculosis caused by the R1Rv strain are unable to cause progressive disease in normal animals; moreover, the bacilli remain localized in the tissues altered by the silica dust and do not give rise to generalized invasion. Similarly, silicosis in man increases the severity of pulmonary tuberculosis but does not increase hematogenous spread of the disease. In other words, silicosis facilitates infection either by causing the *local* accumulation of substances that favor the growth of the bacilli or by interfering *locally* with the mobilization or performance of the defense mechanisms which would otherwise hold bacillary multiplication in check.

At the present time nothing is known of the comparative susceptibilities of virulent and attenuated strains of tubercle bacilli to the immunologic mechanisms that operate in tuberculosis but there is some suggestion that the attenuated forms are more susceptible than the virulant to the bacteriostatic effect of certain organic acids. Since these products accumulate in inflammatory and caseous areas, it appears worth considering that the physicochemical phenomena which accompany inflammation and necrosis may play a part in determining the invasiveness of different strains of tubercle bacilli.

HEMAGGLUTINATION TEST

Like other infectious agents, tubercle bacilli elicit in infected individuals the production of a multiplicity of antibodies directed against the different bacillary components (carbohydrates, proteins, etc.). These antibodies can be readily detected and measured by several types of laboratory procedures (agglutination, precipitation, complement fixation, etc.) but despite much effort no convincing evidence has yet been adduced that they can be of help in determining the degree of resistance to infection or the prognosis of the disease. During recent years a technical innovation has been introduced which promises to increase somewhat the practical usefulness of serologic reactions in following the course of the disease.

When sheep erythrocytes are placed in contact with certain aqueous extracts of tubercle bacilli, they adsorb on their surface one or several bacillary components which render them agglutinable by the serum of tuberculous animals or patients.[20,34,35,43,45,47] The bacillary component which "sensitizes" erythrocytes to serum agglutination has not yet been identified but it is known that the active material is present in most batches of Old Tuberculin. Indeed, OT has been used as a source of sensitizing antigen by most workers who have applied the hemagglutination test to clinical studies.

Two modifications of the test have been worked out; one in which the patient's own erythrocytes are used instead of sheep cells; another in which hemolysis takes place as a result of addition of guinea pig complement to the sensitized erythrocyte-immune serum mixture. Information is still insufficient to evaluate the comparative merits of these different modifications.[35]

According to present indications the hemagglutination test exhibits a degree of specificity as good as can be expected from an immunologic reaction. Of tuberculin-negative individuals (with or without other diseases) so far tested by four independent groups of workers, 95 per cent gave a completely negative hemagglutination test and all of the other also became negative when their serum was diluted 1:8. Moreover, among tuberculin-positive individuals without signs or symptoms of active tuberculosis (with or without syphilis, hyperglobu-

linemia, lupus erythematosus, etc.) 90 per cent gave a negative test when their serum was diluted 1.8. By contrast, most individuals exhibiting active tuberculous disease gave a positive hemagglutination test at dilutions of serum 1:8 or higher, active sera often reacting in very high dilutions.

Although evaluation of the clinical usefulness of the test must wait upon the analysis of much larger numbers of cases, and even more upon the prolonged study of individual patients in order to compare the changes in serum titer with the evolution of the disease, theoretic considerations appear to justify the following statements:

First, it must be emphasized that the antigen responsible for the hemagglutination test has not yet been isolated in a state of purity and that there exist in tubercle bacilli antigenic components which exhibit cross immunologic reactions with those of other microbial species. It is not surprising, therefore, that certain crude extracts of tubercle bacilli have been found to give a high percentage of positive hemagglutination tests in non-tuberculous patients, and it is certain that the specificity of the test depends in no small measure upon adequate selection of the antigen. Unfortunately, this selection will have to remain purely empirical until the active component has been better identified chemically; and despite the satisfactory results obtained so far with certain preparations of OT, the use of this material should be considered as a temporary makeshift.

Although ordinary preparations of OT can be used for the hemagglutination test as well as for the skin tuberculin test, these two tests are entirely different in their mechanisms and depend upon two different components of tubercle bacilli, both present in OT. The hypersensitive state to tuberculin, once it has been induced, is lasting. Indeed, it may persist for several years without any new contact with tubercle bacilli or without any evidence that the bacilli are multiplying in the body. A positive tuberculin test constitutes, therefore, an indication that the individual has once been exposed to tuberculous infection but it does not indicate that tubercle bacilli are active in this individual.

As far as can be judged from the very limited knowledge available at the present time, the antibody responsible for the hemagglutination test does not persist long at a high titer in the body in the absence of infection. Its presence in high titer constitutes suggestive evidence that active infection is present or has occurred recently. In this respect the test can be used by the physician as an additional index for determining changes in tuberculous activity. But it must be kept in mind that a negative hemagglutination test, or a decreasing serum titer, does not necessarily indicate arrest of the disease. Thus the test usually becomes negative in very sick patients (this is not surprising as many immunologic reactions become negative in severe illnesses). Furthermore, it is possible to conceive, and it is indeed likely, that the antigenic stimulus which produces the antibody responsible for the hemagglutination test ceases to operate when the bacilli stop multiplying and spreading. Arrest of bacterial proliferation, however, does not mean death of the bacilli, and it is known that the latter can survive for long periods of time in the caseous and inflammatory areas and initiate a new spread of the disease as soon as conditions become favorable to them.

VACCINATION

None of the known antibodies detectable by serological reactions—and this includes the antibody responsible for the hemagglutination test—have ever been shown to play a part in resistance to tuberculosis. And yet it is known that a fairly effective level of immunity can follow recovery from tuberculous infection or be induced by different vaccination techniques. Although the subject of immunity and its mechanisms will be discussed by Dr. Lurie[58] it may be mentioned here that the resistance of experimental animals and man to tuberculosis can be increased by vaccinating with (1) suspensions of virulent bacilli killed by heat or

ultra-violet radiation,[37,53] and (2) living cultures of strains so attenuated as to be incapable of producing progressive disease. Most widely studied among the latter is the BCG strain, a bovine strain which lost virulence in the course of prolonged cultivation in bile-potato medium.[3,11,23] As will be pointed out later the different subcultures of BCG which are available in the world vary somewhat in their level of attenuated virulence. BCG has been reported to produce fatal disease in silicotic experimental guinea pigs but all evidence indicates that it can give rise only to self-limited lesions in normal animals and man. During recent years experimental vaccination has also been carried out with a murine strain isolated from natural outbreaks of tuberculosis in field mice (voles).[52,54] Like the BCG culture, the vole bacillus produces only non-progressive lesions in guinea pigs, cattle and man but the severity of these lesions differs markedly from strain to strain.

It is generally agreed that the state of resistance induced by BCG and the vole bacillus depends upon the fact that these organisms are capable of some degree of proliferation in the tissues of the vaccinated individual. One may assume, therefore, that the effectiveness of vaccination depends, in a certain measure at least, upon the factors which control the extent to which bacillary multiplication takes place *in vivo*. These factors can be considered under several headings:

1. It has been shown by animal experimentation and by observations in man that individuals differ markedly in their susceptibility to BCG.[29,50] The severity and duration of the pulmonary lesions produced in mice following intravenous injection of the culture, or the skin lesions produced in guinea pigs, rabbits and man by intradermal injection are determined in part by the extent of bacillary multiplication. And there is no doubt that great variations in the extent and duration of the lesions occur from one individual to the other.

2. The number of living bacilli injected is naturally of importance. It is the common practice to express the amount of vaccine injected in terms of mg. or cc. of a given preparation. But it must be realized that the number of *living* organisms in any preparation decreases rapidly as the material ages and becomes very small after the vaccine has been kept for longer than a few days at room temperature. As the immunizing activity of the vaccine depends not upon the amount of bacillary material injected but upon the number of living cells that it contains, it is entirely meaningless and, indeed, misleading to prescribe the dose of vaccine in mg. or cc., as these weight and volume units give no idea whatever of the number of living organisms present in the preparation used. The same limitation would apply to any vaccine consisting of living organisms. Standardization and control of vaccination with BCG or the vole bacillus will be greatly facilitated, therefore, when there are available vaccine preparations rendered more stable by cultivation of the organisms in new types of culture media, and preferably by desiccation with technics that do not destroy the viability of the bacterial cells.[19]

3. There are clear indications that the various cultures of murine type isolated from voles differ somewhat in their virulence for other mammalian species. Similarly, the different substrains of BCG also differ in their intrinsic degree of attenuation of virulence, and this despite the fact that they are all derived from the same bovine culture. When given numbers of living BCG bacilli are injected under identical conditions into uniform animals of a given breed, the severity and duration of the lesion (in the lungs or in the skin) will vary with the BCG substrain used. It is, therefore, desirable that the degree of attenuated virulence of the culture be defined with greater precision since this factor certainly affects the extent of multiplication of the vaccine in man and, consequently, its immunizing effect.

4. In addition to the fact that the different BCG substrains differ in their intrinsic

level of attenuation of virulence there is the further difficulty that each BCG culture is made up of a heterogeneous cellular population. When dilutions of BCG cultures are plated on oleic acid-albumin agar, one always obtains several colonial types some of which exhibit the serpentine pattern of the virulent cultures, others the unoriented mode of growth of the avirulent variants, and the largest percentage, an intermediate type of colonial morphology. These different colonial types can be isolated and maintained in a state of relative purity by repeated transfers in liquid or on solid media, a fact which shows that they do not correspond to cultural artifacts. There is no indication that this type of colonial variation is reflected in different levels of virulence, and none of the forms thus far isolated has proved capable of producing progressive disease in normal animals. But since the different colonial forms must differ in certain characteristics other than their morphology, it would seem advisable to determine their comparative immunogenic properties in order to standardize further the preparation of the vaccine.

Thus several technical aspects of vaccination with BCG or with the vole bacillus render difficult an effective standardization of the procedure and it is not unlikely that the lack of uniformity of the results obtained with the BCG vaccine originate from the fact that bacterial suspensions of very different levels of activity (qualitative and quantitative) have been used for vaccination. Even more regrettable is the lack of methods for evaluating the results of vaccination in terms of immunity to the disease. At the present time conversion of the vaccinated individual from the tuberculin-negative to the tuberculin-positive state is the only criterion available for determining whether the vaccine has "taken." But the tuberculin hypersensitiveness induced by BCG is, at best, of a low order and difficult to evaluate quantitatively. Moreover, the tuberculin test measures allergy, not immunity, and it is known that the levels of allergy and of immunity follow independent courses. For example, guinea pigs vaccinated with heat-killed avirulent in admixture with bacilli adjuvants can develop a level of tuberculin skin allergy higher than that obtained with BCG vaccination, without their resistance to infection being increased to a comparable degree. The tuberculin test, therefore, cannot be used as an index of resistance to tuberculous disease.

CONCLUSION

It seems worth emphasizing, in conclusion, that the lack of technics for measuring immunity in tuberculosis, and the ignorance of the mechanisms of physiologic and immunologic resistance handicap all those concerned with the study and control of the disease: the pathologist wishing to understand how the bacilli establish themselves in tissues and how the latter get rid of the infection; the physician attempting to evaluate the resistance of his patient in order to devise a rational method of therapy; the public health officer conducting epidemiologic surveys or a vaccination program. The most that can be said is that two independent categories of factors play a part in resistance to tuberculosis, one determined by physiologic-biochemical processes, the other dependent on specific immune reactions conditioned by prior contact with tubercle bacilli. But knowledge is still insufficient to identify these factors or to evaluate their activity and comparative importance.

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Native and Acquired Resistance to Tuberculosis*

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THAT a first infection confers increased resistance to a subsequent infection is one of the best established principles in tuberculosis research. However, the immunity acquired is only relative, not absolute as in smallpox. In tuberculosis "the acquired resistance is only a specific increment of the natural resistance."[1] It is incapable of transforming a susceptible species or organ into one that is completely immune.[2] It is desirable, therefore, to consider to some extent the nature of natural resistance.

NATURAL RESISTANCE

It is well known that rats are highly resistant to tuberculosis, yet the tubercle bacillus multiplies readily and persistently within their bodies.[3] However, they develop but slight sensitivity to tuberculin; and although their lesions may be extensive, caseation and softening do not occur.[4] The tubercle bacillus lives almost in symbiosis with the rat. Of a somewhat different nature is the resistance of the chicken to bovine or human bacilli. Bovine tubercle bacilli multiply only to a limited extent in the body of the fowl[5] due to the fact that bovine bacilli multiply meagerly at the body temperature of the chicken. Rich and McKee[6] and Enders and Shaffer[6] have shown that one factor in the immunity of rabbits to Type III pneumococcus is the rapid development of a temperature of 104° to 106°F. (40° to 41.1°C.) in which these pneumococci die.

It is common knowledge that rabbits are susceptible to the bovine bacillus and resistant to the human bacillus. By the use of cultural methods[7] it was found that at first human tubercle bacilli readily multiply in the rabbit. However, although the human bacillus is soon destroyed by an acquired resistance and the animal recovers, the bovine bacillus continues to multiply in the lung and kidney until the rabbit succumbs to the disease. Thus in the slowly progressive disease of tuberculosis it can be seen that what appears on the surface as the native resistance of the rabbit to human type tubercle bacilli really results from a specific resistance acquired during the progress of the disease, for at first no effective opposition to multiplication of this organism exists.

The natural resistance of human beings to tuberculosis bears a certain relationship to the resistance of the rabbit to the human type tubercle bacillus. The vast majority of civilized mankind completely recovers from its primary infection just like the rabbit. There is no race of human beings which is completely immune to tuberculosis. However, the mortality and morbidity from tuberculosis of different races varies widely. Whether these are due to true genotypic, inherited differences in native susceptibility and immunizability or to other factors is still widely debated.

Diehl and von Verschuer[8] studied tuberculosis in identical and fraternal twins. The former have the same genetic determinants; the latter are genetically different. Of thirty-seven identical twins, twenty-six behaved in similar fashion toward tuberculosis. In the remaining identical twins the tuber-

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culosis pursued an unlike course. Thus there was 70 per cent correspondence between the genetic constitution and the behavior toward tuberculosis. On the other hand, among sixty-nine fraternal, i.e., genetically different, twins the tuberculosis was of the same nature in seventeen and of a different character in fifty-two twins; the correspondence in this group was, therefore, only 25 per cent. From these studies the authors conclude that heredity plays a deciding role in tuberculosis. Kallmann and Reisner[9] used the "Twin Family Method" which differs from the twin study previously cited in that not only is the tuberculous morbidity of mono-ovular and bi-ovular twins compared but also that of various sibship groups as well as of the marriage partners of the twin index cases. They found that the chance of developing tuberculosis increased in strict proportion to the degree of blood relationship to a tuberculous index case. They believe that these consistent differences could not be explained by environmental factors.

Plainly, therefore, studies in human beings remain difficult to interpret, for environmental factors cannot be excluded. For this reason Wright and Lewis[10] studied the resistance of guinea pig families that had been inbred for about fourteen generations by brother and sister mating. They found that with subcutaneous injection of a standard dose of tubercle bacilli the difference in longevity was as much as 40 per cent. It is evident from their studies, as critically analyzed by Hill,[11] that one family is genetically endowed with a considerably greater resistance to tuberculosis than the others. In further studies Lewis and Loomis[12] correlated this increased resistance to tuberculosis of guinea pig families with their innate capacity to form antibodies. They found that the most resistant families produced a greater amount of hemolysins and agglutinins when exposed to their antigens.

Lurie[13] has continued these studies in rabbits. By brother and sister inbreeding of rabbit groups for ten to twelve generations, families have been developed which exhibit varying inherited, specific resistance to tuberculosis. This has been determined by exposing succeeding generations of the different rabbit families to artificially infected rabbits or to the inhalation of known numbers of bacilli. It was found after excluding all known environmental factors that resistance to tuberculosis is a function of the genetic constitution of the rabbit. The genetic constitution *per se*[14] determines whether, under given conditions of natural respiratory contagion, rabbits will acquire (1) a rapidly progressive, primary, generalized tuberculosis resembling that seen by Borrel in Singalese troops, (2) a localized, chronic, ulcerative pulmonary phthisis analogous to the "reinfection" type of tuberculosis in white adults, or (3) a disease of a character intermediate between these extremes, as seen in the American Negro.

The fundamental variant of the disease developed by these families is the degree of localization of the infection at the portal of entry. This localization in turn is a function of the rapidity and intensity of the development of local immunity in the primary pulmonary focus. The mononuclears of the most resistant family rapidly acquire an effective capacity to inhibit the growth of tubercle bacilli in their cytoplasm. As a result progress of the disease at the portal of entry is slow. Soon the focus becomes encapsulated and undergoes liquefaction. The bacilli focalized by lymphogenous or hematogenous routes in the lymph nodes or internal organs are prevented from multiplying. Dissemination of the disease takes place chiefly by contiguity or tubular spread of the softened material in which the bacilli multiply. The rate and intensity of development of local immunity in the families of least resistance were low and feeble. The mononuclears did not acquire any considerable capacity to inhibit the growth of the bacilli in their cytoplasm. As a result the primary focus progressed fulminatingly and did not become encapsulated. Dissemination and multiplication of the bacilli took place in the organs focalized by lymphogenous and hematogenous routes.

Recently, by the use of the method of natural quantitative airborne contagion[15,16] through which animals can be exposed to the inhalation of a known number of tubercle bacilli at a single sitting, it was shown that with certain doses of inhaled human type tubercle bacilli and at a given time after infection an extensive tuberculosis pervades the lungs of susceptible rabbits while no tuberculosis at all is found in resistant strains.[17] This difference is due to the fact that the lung of the resistant rabbit soon destroys the human type tubercle bacilli while the lungs of susceptible animals allow them to multiply for a long time. Under these conditions tuberculin sensitivity develops more rapidly in the resistant animals. Thus although the difference in response of susceptible and resistant rabbits to bovine tubercle bacilli is only one of degree, their reaction to the less virulent human organisms results in an all-or-none effect.

These observations suggested that the response of animals to BCG, a strain of tubercle bacilli which never causes progressive disease, might serve as an index to the natural resistance to tuberculosis. It was found that the nodule at the site of intracutaneous inoculation in the resistant rabbit grows rapidly, reaches its peak quickly, tends to ulcerate and heals soon. In the susceptible rabbit this nodule grows slowly, reaches its peak tardily, does not usually ulcerate and heals much later.[18] The BCG multiplies in the skin of the resistant rabbit for a shorter time and is subsequently more rapidly destroyed than in the susceptible rabbit. As a consequence allergic sensitivity to tuberculin develops more rapidly and intensely in the resistant animal. Clearly, whole bacilli cannot sensitize; only on the disintegration of the BCG and the release of its antigens can allergy develop. Likewise antibodies against the tubercle bacillus appear more rapidly and in higher titer in the resistant animal. Thus the varying response of genetically resistant and susceptible rabbits to the highly virulent bovine type bacillus, to the human bacillus of lower virulence for the rabbit and to the BCG of least virulence is characterized by correspondingly graded differences in the uniform reactions of each race. There is no inherent reason why the response of human beings to the intracutaneous inoculation of BCG should not be an accurate index to the native resistance of different individuals to the disease. This seems likely as it is now apparent that man on first infection with the tubercle bacillus, like rabbits of different genetic resistance, may develop either the so-called reinfection type of disease with little or no tracheobronchial involvement or the so-called primary type with extensive disease in the hilar nodes.[19,20]

As to the physiologic basis of this varying inherited local and general resistance, it may be said that in the two most sharply and uniformly contrasting families low skin permeability to particulate matter, rapid and intense development of allergic sensitivity and rapid and high production of antibodies were associated with high resistance; high skin permeability, slow and feeble development of allergic sensitivity and tardy and low production of antibodies were associated with low resistance.

It has been further shown that hereditary resistance to natural airborne contagion of tuberculosis has two phases.[21] Resistance to being naturally attacked by the tubercle bacillus is distinct from resistance to the ensuing disease. One inbred family has little resistance against being attacked by this contagion but great resistance against progress of the ensuing disease. Another inbred family has greater resistance against the engrafting of the infection but little resistance against dissemination of the disease after it has taken root.

Since it was known that estrogen reduces skin permeability[22] and that chorionic gonadotropin, by inducing corpora lutea in the ovaries, increases this permeability,[23] the role of these sex hormones in genetic resistance was investigated.[24] It was found that by subjecting highly inbred susceptible rabbits to estrogen their resistance to an intracutaneous infection could be con-

siderably increased. On the other hand, by exposing resistant animals periodically to chorionic gonadotropin their resistance could be materially diminished. However, the opposite effects of these two hormones resulted not from their different influence on the essential mechanisms of natural resistance, i.e., the rate of multiplication and destruction of tubercle bacilli in the tissues, their allergic irritability or their capacity for antibody production, but through their effects on connective tissue and vascular permeability. Estrogen, by reducing these permeabilities, tends to retard spread of the disease in the body and in a manner unknown protects parenchymatous organs against amyloid degeneration. On the other hand, chorionic gonadotropin via its ovarian consequences increases these permeabilities and enhances dissemination of the infection.

It was noted in these studies that tuberculosis in the rabbit is accompanied with marked hypertrophy of the adrenal cortex. Furthermore, this hypertrophy is proportional, at least to some extent, to the native resistance of the animal.[25] Evidence has also been accumulating which suggests that the adrenal may play an important role in phagocytosis[26] and, via its effect on lymphocytes,[27] in allergic sensitivity and antibody production. It is noteworthy that the adrenals of genetically resistant rabbits are more responsive to stressing stimuli such as cold than those of susceptible animals. An investigation into the role of the adrenals in constitutional resistance is now being actively pursued.

It is clear then that hereditary, constitutional determinants of resistance are numerous and synergestic.

The environmental variable, the intensity of natural contagion,[21] may exercise an important influence both on the incidence and type of disease acquired by these families. At low intensities a small proportion of the least resistant rabbits acquire a fatal tuberculosis while the most resistant family similarly exposed escapes all evidence of disease except for the acquisition of an evanescent tuberculin allergy. High or low intensities of contagion do not change the type of disease acquired by the families of low resistance. It is always fulminating and maximal. In the family of high resistance, however, not only does a high intensity of contagion increase the incidence of the acquired disease but also it modifies the genetically controlled response to the infection to such a degree that the disease is of intermediate type sharing some of the characters of the infection in rabbits of high and low resistance. There is some evidence that diet may affect this genetic resistance.[28]

ACQUIRED RESISTANCE

All investigations on acquired immunity to tuberculosis date from the fundamental observation of Koch[29] who in 1891 described what is known as the Koch phenomenon. It is evident from this phenomenon that to the tuberculous animal the tubercle bacillus is a violent poison; to the normal animal, on the other hand, it is at first almost innocuous. Furthermore and paradoxically, this hypersensitivity to the tubercle bacillus is accompanied with a manifest relative immunity, for although the tissues at the site of local inoculation die, the internal organs are spared for a long time and the regional nodes do not become involved.

Allergy and Immunity. For many years the most widespread opinion has been that allergy, or the capacity of the tissues of the infected animal to react with exaggerated and accelerated acute inflammation to reinfection, is the essential mechanism of the acquired immunity. This was based on the observation that in general there is a parallelism between the two phenomena. Natural and artificial procedures which produce no hypersensitivity to tuberculin do not produce any immunity to tuberculosis. In this country the outstanding work of Trudeau,[30] Baldwin[31] and particularly the investigations of Krause[32] have emphasized the parallelism between allergy and immunity so that one readily believed with Krause that immunity is a function of allergy.

What is the fate of the bacilli of reinfection? In Koch's phenomenon most of the bacilli are extruded from the body together with the slough,[33] but obviously this is a highly unnatural phenomenon. If the site of reinfection does not undergo necrosis, as occurs with small dosage, and if this site is inoculated into susceptible animals, it was found by Römer[34] and by Paterson[35] that the bacilli persist in the tissues in a virulent form.

In 1924 Opie[36] published his important investigations on the Arthus phenomenon. Foreign proteins such as crystalline egg albumin introduced into the skin of a normal rabbit produce slight inflammation, spread widely and soon enter the blood stream. In an animal sensitized to it the introduced protein produces a severe inflammation, remains localized and fails to enter the circulation. Similar observations have been made by Krause and Willis[37,38] in experimental tuberculosis. If the excised lymph nodes draining the site of inoculation of a normal animal are injected into guinea pigs, it can be shown that the bacilli reach these nodes within twenty-four hours. In the reinfected animals it requires two to three weeks for the bacilli of reinfection to pass to these nodes. Since no specific bacteriolysins have been demonstrated in tuberculosis, Krause[39] attributed the fixation of the bacteria at the site of reinfection to the barrier of heightened acute inflammation or allergy. Krause and Peters[40] have also demonstrated that a tuberculous animal reacts with accelerated tubercle formation, and tubercles have been regarded as a factor in limiting the spread of bacilli.

In 1929 Rich and McCordock[41] pointed out that there is really no direct evidence that the death of tissue and the exaggerated acute inflammation which characterize the allergic reaction are in themselves responsible either for the fixation of the bacilli or for inhibition of their multiplication. In a series of interesting investigations Rich and his associates have attempted to separate allergy from immunity. From the standpoint of the present discussion, the most significant observation is that of Rothschild and his associates[42] who rendered guinea pigs hypersensitive to tuberculin by vaccination with an R-1 culture; when allergy was well established, they desensitized these animals by administration of increasing doses of tuberculin until no reaction occurred to the injection of as much as 1 cc. of undiluted tuberculin. These vaccinated and desensitized animals were then given a test dose of virulent tubercle bacilli. Desensitization was continued throughout the course of this disease. It was found that despite the continued absence of allergy these desensitized animals showed a degree of immunity in no way different from guinea pigs similarly immunized but with allergy unaffected by desensitization. These observations have been amply confirmed.

However, Willis and his associates[43] have repeated the experiments of Rothschild[42] but, instead of killing the test animals, allowed them to die. Under these conditions the allergic animals that had been desensitized with tuberculin and maintained in this state for the course of the experiment lived a shorter time, showed more extensive pulmonary disease and contained more tubercle bacilli in their tissues, as demonstrated both by culture and by direct smear, than the guinea pigs that had not been desensitized. It is difficult to state to what extent the results of this treatment with tuberculin are due to the removal of the exaggerated inflammatory responsiveness of the tissues to the tubercle bacillus; it is clear, however, that desensitization with tuberculin, possibly because of the associated malnutrition and adrenocortical exhaustion,[26] interferes with the enhanced capacity of mononuclear phagocytes to destroy or inhibit the growth of tubercle bacilli in them which they acquire as a result of a primary infection. It is noteworthy in this relation that Steinbach[44] demonstrated that sensitized guinea pigs may be protected from tuberculin shock with ascorbic acid which is discharged from the adrenal during the physiologic action of its cortical hormones.

It would seem, therefore, that acute, excessive allergic inflammation is not essential to the operation of immunity. Does inflammation assist in the fixation of bacteria at the site of introduction? In an extensive series of papers Menkin[45] has shown that inflammation has the capacity of fixing *in situ* a variety of substances including bacteria. This, he maintains, is brought about by the coagulated plasma of the exudate and by thrombosed lymphatics that occur at the site of prepared inflammation. It has been shown by Lurie[46] that at the site of tuberculous reinfection in immunized rabbits a greater barrier of fibrin is deposited than in the normal animal. However, with large reinfecting doses incorporated in melted agar and trypan blue the increased lymph flow resulting from the intensified inflammation in the immunized animal brings about a more rapid dissemination of the bacilli, the agar particles and the trypan blue to the draining lymph nodes than in the normal animal, despite the greater barrier in the former. Living tubercle bacilli were demonstrated in the lymph nodes draining the site of reinfection at a time when the regional nodes of the normal animal were still sterile. On the other hand, in guinea pigs, even with large reinfecting doses the bacilli, agar particles and trypan blue are retarded in their dissemination.[47] It was found by Menkin[48] that the fixation of trypan blue at the site of inflammation depends upon the character of the irritant. The powerful necrotizing agent of Staphylococcus aureus produces rapid fixation. Mild irritants, on the other hand, produce only delayed fixation. Now it is well known that guinea pigs develop a far greater sensitivity to tuberculin than do rabbits. In the former the tuberculin reaction often proceeds to necrosis; in the latter this is rarely seen. It is obvious that the inflammation caused by the tubercle bacillus of reinfection in the rabbit is mild and hence is incapable of fixing the bacilli of reinfection. On the other hand, in the guinea pig and especially in the much more highly sensitive human being[49] the exaggerated inflammation may be of such a character as to aid in the fixation. In fact a comparative study of the site of reinfection in rabbits and guinea pigs[50] has revealed significant differences in their character. The lymphatics surrounding the focus of reinfection in the rabbit remain patent and thus apparently permit the passage of tubercle bacilli to the draining lymph nodes. In the guinea pig, however, these same lymph vessels become thrombosed by a delicate and intimate network of fibrin and thus, perhaps, interfere with the ready passage of bacteria.

Rich believes that the fixation of bacilli in the immune animal is a function of the antibody. It has been shown by Mudd[51] and his co-workers that when bacteria are coated with antibody they adhere to each other and to leukocytes with which they come in contact. Rich[52] has shown that in the normal animal virulent bacteria grow dispersed in the tissues. In the immune animal they adhere to each other and therefore grow in clumps in the tissues. They also adhere to fibrin shreds and other tissue elements. This effect of antibody tends to immobilize the bacteria. A similar *in vivo* agglutination has been shown by Lurie[46] to occur in tuberculous reinfection.

Although Rich[53] and his school accept the localizing capacity of an existing inflammation for certain bacteria, they question whether the allergic inflammation in tuberculosis sets in with sufficient rapidity to prevent the dissemination of the bacilli of reinfection. Opie[54] has shown that if carbon particles, staphylococci or tubercle bacilli are suspended in citrated plasma, and if this plasma is coagulated by the addition of calcium, the particles and bacteria become enmeshed in the fibrin network during the process of clot formation while the remaining serum is freed from both. This process may be essential in the mechanism of fixation, the thrombi forming in the lymphatics merely aiding this process. It is likely, therefore, that early in the stage of an allergic inflammation associated with considerable tissue injury the thrombokinase released will quickly clot the exudate poured

out of the blood vessels and thus remove the bacilli from the fluid that drains away from the site of reinfection. That this cell injury occurs within an hour after reinfection would follow from the work of Favour and his associates which is discussed hereafter. The localization of non-specific agar particles and trypan blue at the site of reinfection in sensitized guinea pigs cannot be attributed to antibody action; hence the allergic inflammatory process *per se* mechanically aids fixation. It is concluded, therefore, that an intense allergic inflammation is an aid in the fixation of the bacilli at the site of reinfection.

The fixation of foreign protein in the Arthus phenomenon is in part due to the precipitating effect of the antibody. However, Opie[55] is of the opinion that both in the case of fixation of foreign protein and in the immune reactions associated with inflammation, as in tuberculosis, the relative importance of antibody and inflammation cannot be estimated (both favor fixation), for allergic inflammation brings to the site with greater readiness and in greater amount all the elements of an inflammatory exudate including antibodies, fibrin, granulocytes and macrophages. These processes fix foreign proteins, bacterial products and bacteria themselves at the site of inflammation so that their penetration into the blood stream is retarded.

It is obvious that the antigenic consistency of the tubercle bacillus is complex. Desensitization to allergic inflammation is compatible with immunity if the two are referable to different antigenic components. There is much evidence to suggest that the allergic inflammation is due, in part at least, to the protein as it occurs within the tubercle bacillus in the tuberculous lesions. In experimental tuberculosis in the rabbit Freund and his co-workers[56] found sensitivity to tuberculin subject to considerable variation but the titer of complement fixation remained constant. Enders[57] observed that guinea pigs that had been sensitized with killed tubercle bacilli and that had survived anaphylactic shock to the carbohydrate fraction of the tubercle bacillus show no diminished skin sensitivity to tuberculin. Furthermore, Petroff and his co-workers[58] found that heat-killed avirulent, rough "R," avian tubercle bacilli produce sensitization and some immunity in chickens. Virulent and smooth "S" strains produce more immunity and less sensitization.

Most recently new light has been shed on this phenomenon. Choucroun[59] extracted potent antigenic substances from tubercle bacilli with mineral oil. Middlebrook et al.[60] noted that virulent tubercle bacilli usually grow in skeins or cords due to the fact that on division the daughter cells of a single rod adhere to each other along their long axis. Avirulent bacilli, on the other hand, do not characteristically form such cords but grow in a more or less unoriented fashion. Bloch[61] has continued these studies and has demonstrated that this adhesion of virulent bacilli to each other is due to a lipid on their surface which is soluble in petroleum ether. If virulent bacilli are treated with this hydrocarbon, their viability is not affected but their virulence is reduced. It had previously been found by Allgöwer and Bloch[62] that the motility of phagocytes that had ingested virulent tubercle bacilli is markedly inhibited whereas phagocytes that had ingested avirulent tubercle bacilli are not injured. Treatment of virulent bacilli with petroleum ether robs them of this toxic effect on leukocytes. It is surmised that this lipid may be a determinant of virulence.[63] It is noteworthy that this lipid does not induce tuberculin sensitivity in normal animals nor does it elicit the tuberculin reaction in tuberculous individuals.

Thus sensitization and immunity may be referable to different antigenic agents and no strict parallel need be present between them. It will be shown later that the chief mechanism of immunity is the increased capacity of the mononuclear phagocytes to inhibit the growth of tubercle bacilli. Allergic inflammation is not essential for this process. It is believed, however, that under certain conditions it may aid in the protection.

Nature of Allergy. The profound influence that allergy exerts on the development of the disease warrants its consideration. The tuberculin reaction bears a certain relationship to anaphylactic sensitization to foreign protein, as seen in the Arthus phenomenon. The two conditions, however, are distinct in important respects. Tuberculin sensitivity cannot be induced by the injection of tuberculin. It is conditioned by the presence of tuberculous lesions and varies in intensity with the extent of these;[64] yet Raffel[65] has recently produced delayed tuberculin hypersensitivity by means of the protein plus the wax of the tubercle bacillus. It is noteworthy that this wax induces the formation of epithelioid cells.

Anaphylactic sensitization to tuberculoprotein is independent of such lesions. Baldwin[66] showed that tuberculous guinea pigs occasionally but not uniformly are killed in true anaphylactic shock by the administration of tuberculoprotein. Injection of tuberculoprotein may sensitize normal animals to the tuberculoprotein but not to tuberculin. Austrian[67] demonstrated that sensitivity to the protein of the tubercle bacillus can be transferred to normal animals. There is no conclusive evidence that tuberculin sensitivity can be transferred to a normal animal by humoral substances. Even by the Prausnitz-Küstner technic Coca and Grove[68] failed to transfer this sensitivity. The skin reaction in tuberculin sensitivity appears after a delay of several hours and reaches its maximum development in forty-eight hours. Protein sensitivity of the skin is immediate in appearance and often transitory. Lewis and Seibert[69] and Seibert,[70] by the use of proteins prepared from culture filtrates of tubercle bacilli grown on synthetic media, have demonstrated that such tuberculoproteins are excellent antigens and produce all the classical reactions of anaphylactic sensitization both in rabbits and guinea pigs.

In common with Baldwin[71] and many other investigators Seibert found that rabbits sensitized to the protein of the tubercle bacillus react slightly or not at all to tuberculin. Krause[72] demonstrated that tuberculin sensitivity is associated with some degree of immunity to reinfection. Anaphylactic sensitization to tuberculoprotein, however, develops entirely independently of immunity. Seibert[71] confirmed this observation and has shown that guinea pigs sensitized to tuberculoprotein, far from being protected against infection, actually develop more destructive lesions than the unsensitized controls.

A number of observers[74] have noted that in tuberculin sensitivity the cells are injured on contact with tuberculin. Rich and Lewis[75] clearly demonstrated by tissue culture that the addition of tuberculin to explants of leukocytes or spleen of tuberculous animals inhibits their growth and migration even in the presence of normal serum, whereas to cells derived from normal animals tuberculin is innocuous, even if cultured in tuberculous sera. Aronson[76] confirmed and extended these observations. That circulating antibodies are completely ruled out from these effects and that it is the cells themselves that are sensitized to tuberculin in tuberculous animals has been shown by Moen and Swift[77] who found that this sensitivity to tuberculin is retained by the cells after several transplants in tissue culture.

In 1942 Landsteiner and Chase[78] in studies on experimental drug allergy found that specific hypersensitivity of the delayed type is transferable to normal guinea pigs by means of exudate cells derived from sensitized animals. This was climaxed by the success of Chase[79] in transferring tuberculin sensitivity from sensitized to normal guinea pigs within two to three days after the intravenous or intraperitoneal injection of exudative mononuclear cells, lymphatic or splenic tissue derived from sensitized animals.

With anaphylactic sensitization, on the other hand, and easily demonstrable circulating antibodies, the consensus is that the cells are not injured *in vitro* on addition of the antigen.[80] Meyer and Loewenthal[81] and Aronson[81] have shown that the addition of horse serum to transplants of tissues derived

from guinea pigs sensitized to it has no inhibitory effect on the growth of these cells, even when the cells are cultured in serum containing large amounts of homologous precipitins. Furthermore, in anaphylactic sensitization as in the Arthus phenomenon not only can this sensitization be passively transferred to normal animals by the introduction of the precipitin-containing serum but also, as has been shown by Opie and Furth,[82] inflammation will result in a normal animal by the procedure of reverse anaphylaxis, i.e., by introducing the antigen first and the antibody-containing serum later. In view of this observation it is clear that one need not assume any sensitization of the tissues themselves in the operation of the Arthus phenomenon. In fact, Opie[83] has shown that introduction of the washed precipitate resulting from the interaction of antigen and antibody into normal animals results in inflammation. Doerr[84] has stated that the inflammation in the Arthus phenomenon is not due to the action of the antigen on sensitized tissues but to the action of the antigen-antibody precipitate on the surface of or within normal cells. Rich[85] found that the avascular cornea of a rabbit sensitized to horse serum shows little inflammation on injection of the antigen. However, if the cornea had been previously vascularized by a non-specific irritant, introduction of horse serum into this cornea is followed by severe inflammation, with the greatest injury in the endothelium. From this he concludes that in anaphylaxis the site of sensitization is the endothelium of blood vessels. This interpretation cannot explain the severe inflammation in the reverse, passive Arthus reaction in which tissue sensitization of any kind could not come into question. In a word, tuberculin allergy is a property of the sensitized cells while anaphylactic reactions result from the effects of the interaction of antigen and antibody on cells whose sensitization cannot be demonstrated *in vitro*. An alternative interpretation is that the tuberculin reaction results from the interaction of antigen or tuberculin with antibody which is in intimate union with the cells.

An important contribution to the understanding of the nature of bacterial allergy has been made by Dienes and his associates. If a tuberculous guinea pig is sensitized with horse serum or crystalline egg white, it is found on the sixth day that injection of these antigens into the skin produces large necrotic reactions.[86] This reaction has all the characteristics of the tuberculin as distinguished from the anaphylactic type of sensitivity for it is delayed in appearance, of long duration and is not associated with circulating antibodies. Although these animals react with severe necrosis on injection into the skin of a few hundredths of a milligram of horse serum, they will not be thrown into anaphylactic shock by the intravenous injection of a larger amount of this antigen.[87] Later, when precipitins appear in the blood, the animal can be thrown into anaphylactic shock and the sensitivity can be transferred to a normal animal.

Most recently Raffel[88] has shown that instead of using the whole tubercle bacillus as Dienes did, addition of a purified wax of the bacillus was sufficient to transform the reaction to soluble egg albumin from the immediate anaphylactic type of hypersensitivity, unaccompanied with any evidence of sensitization of the cells to the antigen, into the delayed, tuberculin type of hypersensitivity, with evidence of sensitization of the cells to the egg white as demonstrated by tissue culture and the corneal reaction.

Dienes and Mallory[89] have also shown that the inflammation of the tuberculin type of sensitization is associated in its milder form with a predominance of mononuclears, whereas anaphylaxis and passive sensitization are characterized by an edematous and polymorphonuclear infiltration. Laporte[90] confirmed these observations. Other observers have failed to do so.[91]

Zinsser[92] found that tuberculous guinea pigs first develop tuberculin sensitivity and later anaphylactic sensitization to tuberculoprotein. Dienes maintains that in the

process of sensitization with any antigen, whether of bacterial or other origin, the tissues first develop a sensitization without circulating antibodies, and later the anaphylactic type supervenes with the appearance of antibodies in the blood.

Evidence supporting this view was recently furnished by Chase.[93] It has already been pointed out that he had transferred the delayed types of hypersensitivity to normal animals by administering to them cells derived from sensitized donors. If such normal animals are given six- to eightfold the lymphoid cell mass necessary to confer upon them the delayed type of hypersensitivity, the recipients will show circulating antibodies three to four days after this injection. Thus the lymphoid tissue from sensitized animals at first confers upon normal recipients the delayed type of hypersensitivity and later circulating antibodies.

In tuberculosis the pre-anaphylactic type of sensitization develops to a marked degree. According to Chase's conception, in tuberculin sensitivity and in bacterial allergy in general the antibodies, if any are present, are intimately bound to the cell. Therefore, on contact with the antigen the mononuclear cells undergo injury or multiplication, depending on the concentration of the antigen; hence the predominance of mononuclears in the tuberculin type of sensitivity. The immunity in this stage is chiefly local and operates to circumscribe the antigen. It is noteworthy that Lurie[7] found that when immunity sets in the destruction of tubercle bacilli is much more effective in certain organs than in others, suggesting the role of local factors in the immunity. In fact[50] he has shown that on subcutaneous inoculation of tubercle bacilli the micro-organisms undergo destruction first at the portal of entry. The bacilli in the nearest draining lymph nodes are subjected to destruction next while at the same time the bacilli situated in distant metastatic foci, as in the lung or liver, are still multiplying unhindered. In anaphylactic or passive immunity the circulating antibodies operate to neutralize the antigen that has escaped the barrier of local inflammation. Since tuberculosis in man is largely a local disease of the portal of entry, the lung, it is not surprising that the immunity is chiefly of the local type.

Mechanism of the Tuberculin Reaction. Tuberculin sensitivity can be induced by heat-killed tubercle bacilli. This has been known for a long time, for Prudden and Hoddenpyl[94] demonstrated that killed tubercle bacilli produced typical tuberculous lesions with all their pathologic characteristics. The only difference from the lesions induced by living tubercle bacilli is that the former regress and are absorbed.

That tuberculin sensitivity cannot be transferred by humoral antibodies has been noted previously. However, Chase has transferred this tuberculin sensitivity from allergic to normal animals by means of lymphoid cells and his observations have been amply confirmed.[95] It is noteworthy that the sensitivity thus conferred does not appear in the recipient until two to three days after the administration of the cells, that the passively transferred sensitivity is of short duration and that the cells of the sensitized donor lose this property upon heating to 48°C. for fifteen minutes or on freezing. Is it possible that the transferred cells of the donor elaborate the specific antibody necessary for the tuberculin reaction in the recipient host?

A new and extremely delicate *in vitro* tuberculin test has been developed by Favour and his associates. This depends upon the lytic effect of tuberculin on suspensions of white blood cells of sensitized animals. It was first found that lymphocytes of tuberculous mice suspended in their plasma were lysed by tuberculin.[96] Polymorphonuclears of such mice were not injured by the tuberculoprotein. However, both lymphocytes and polymorphonuclears of tuberculous human subjects were lysed by the tuberculin.[97] It was then observed by this delicate technic that lymphocytes of mice adsorb tuberculin *in vitro* while the polymorphonuclears do not. On the other hand, both types of cells derived from

humans adsorb tuberculin.[98] Furthermore, if white blood cells derived from tuberculous humans are thoroughly washed and suspended in plasma derived from tuberculin-negative individuals, no cytolysis occurs on addition of tuberculin.[99] This can be restored by the addition of plasma from an actively tuberculous individual or by the addition of a heat-labile globulin fraction from such plasma.[100] Moreover, if the complement present in tuberculous plasma is removed by the addition to it of an unrelated interacting antigen-antibody system,[101] no lysis of white cells will occur on addition of tuberculin. This lytic effect of the "decomplemented" plasma can now be restored by the addition of complement derived from plasma of a normal, tuberculin-negative individual. Again, the cytolytic effect of tuberculin on white blood cell suspensions of tuberculous animals is greatly enhanced by the injection of tuberculin intracutaneously forty-eight hours before obtaining the white cells.[102] Finally, by incubating lymphocytes selectively obtained from the blood of actively tuberculous individuals with the plasma of tuberculin-negative individuals, and cytolytically inactive, this treated plasma becomes actively cytolytic to white blood cells of normal individuals upon addition of tuberculin.[103]

In interpreting these observations it must be noted that there is no strict parallel between the cytolytic effect of tuberculin on the *in vitro* suspended cells and the skin reaction of the individuals who furnished the cells and plasma.[104] With this provision in mind and awaiting further confirmation there is a strong suggestion in these studies that the tuberculin reaction may result from the interaction of an antibody present in the plasma of tuberculin-positive individuals, which becomes strongly adsorbed to the white cells, with tuberculoprotein, which also has a strong affinity for the same cells. Their interaction leads to the death of a portion of the cells and to the inflammation which is characteristic of the tuberculin reaction.

Thus the demonstration of Rich that tuberculin is specifically toxic to the tissues of a hypersensitive individual, that the body as a whole and not only the site of the lesion becomes hypersensitive, and the new studies of Chase and those of Favour and his associates would suggest that tuberculin allergy is due to the excretion of minute amounts of antibody from the lesions into the circulation and the rapid anchoring of these agents by the cells. Since the role of this antibody has been demonstrated in the *in vitro* test of suspended white blood cells and not yet in the skin reaction, a final statement as to its significance cannot be made.

Role of Humoral Substances in Tuberculosis Immunity. It has been seen that antibodies with an unusual avidity for cellular adsorption may play a role in tuberculin hypersensitivity. Are antibodies of significance in immunity? Precipitins, agglutinins, complement-fixing antibodies and bacteriotropins have been found in the sera of tuberculous individuals. Passive transfer of these sera confers no immunity on susceptible animals. The sera of highly immunized animals have no bactericidal effect upon virulent tubercle bacilli *in vitro*.

Manwaring and Bronfenbrenner[105] could demonstrate no lysins for tubercle bacilli in the blood or exudates of tuberculous animals; but if tubercle bacilli are incubated with bits of omentum of immune animals, some of the bacilli disappear. The authors attribute this in part to lysis of the bacilli by the fixed peritoneal cells. The peritoneum of normal animals is ineffective. The immunologic significance of the recently demonstrated antibodies[106] in the sera of tuberculous individuals with active disease and their absence in the blood of inactive cases has not yet been elucidated. Thus the antibodies that are found in the body fluids of tuberculous animals have not been shown by *in vitro* methods to play any decisive role in immunity to tuberculosis.

Mechanism of Immunity in Tuberculosis. It is clear from what has been presented thus far that the acute, exaggerated allergic inflammation *per se* is not an indispensable factor in immunity to tuberculosis, nor

have humoral antibodies been demonstrated by *in vitro* methods to play a decisive role in the operation of immunity. *In vitro* studies have also failed to bring evidence that the cells acquire an increased capacity to digest tubercle bacilli as a result of infection. Available information had suggested that the bacilli of reinfection as judged by animal inoculation persist in their virulent form at the site of reinfection. This has led certain investigators to assume that the tissues of the immune animal are changed in some subtle way so as to make them an unfavorable soil for the multiplication of the bacilli[41] or, as Selter[107] and Hedvall[108] maintain, that the tissues of the immune animal become indifferent to the presence of the bacilli and do not react with the formation of lesions.

It was previously stated that rabbits, which are naturally resistant to human tubercle bacilli, overcome this infection not by initially inhibiting the growth of the bacilli but by destroying them almost completely after a brisk preliminary multiplication. Lurie[109] correlated the fate of the living bacilli, as indicated by the number that can be cultured, with the cellular reactions of the body. He found that the immediate polymorphonuclear reaction that follows upon the introduction of tubercle bacilli is greater after the more virulent bovine infection than after the human infection, and more in the lung than in the liver, i.e., they were more abundant with the more virulent strain and in the more susceptible organ. Similar and more extended observations have been made by Long and his associates.[110] This would suggest that the virulent organism is initially more toxic to the tissues than the less virulent bacillus. Moreover, it would also intimate that to the natively more resistant organ the toxic principles such, perhaps, as the surface lipids of the bacillus recently demonstrated by Dubos and Bloch[111] are originally less injurious.

Although polymorphonuclears and mononuclears readily phagocytize the bacilli, as has been shown by Mudd and his coworkers[112] even in the absence of immune serum, the granulocytes do not destroy the bacilli; on the contrary these cells die. These dead polymorphonuclears together with their contained bacilli are then taken up by mononuclear phagocytes. Within these phagocytes the bacilli first multiply without any effective opposition, as indicated by the far larger numbers of bacilli that can be cultured from the lesions at this time and by their accumulation within the cells as observed histologically. Woodruff also made similar observations.[113] It is noteworthy that in this first phase the mononuclears are not injured by the bacilli multiplying in their cytoplasm. Some of the bacilli, however, are destroyed at once. As long as the balance between growth and destruction is in favor of the bacilli, the nodule grows larger by accretion on the periphery of new mononuclears coming either from the blood or by mitosis of pre-existing cells. However, after a varying period and synchronous with development of hypersensitivity to tuberculin[114] and the first stages of caseation, the mononuclear cells begin to assume the structure of epithelioid cells and the bacilli begin to diminish in numbers both histologically and by culture. When the tubercle has matured, most of the bacilli, if they are not of excessive virulence, have disappeared. The rapidity with which epithelioid cells are formed varies with the rate of destruction of the bacilli. In two animals of varying natural resistance to tuberculosis the destruction of the bacilli is more rapid in that animal in which epithelioid cells are formed more quickly. The same is true for different organs in the same individual and even for different parts of the same organ. Likewise, as was shown heretofore, genetically resistant rabbits develop allergic sensitivity more rapidly than susceptible animals because of the more rapid disintegration of the bacilli within their mononuclears; hence the more rapid release of the sensitizing antigens of the bacillus.

It has long been established[40] that one of the chief characteristics of the reinfected animal is the capacity for accelerated forma-

tion of tubercle and its abortive nature. It has been shown by Lurie[115] that in the presence of sufficient residual primary lesion the bacilli of reinfection are quickly destroyed without preliminary multiplication, and this increased destruction is associated with a more rapid mobilization of the mononuclear phagocytes, a more rapid formation of these into nodules and a more accelerated transformation of the phagocyte into mature epithelioid cells. Tubercles and epithelioid cells act, therefore, not merely to hem in and imprison the bacilli as is usually maintained but result from the destruction of bacilli. Thus the most conspicuous and characteristic phase of the tuberculous lesion is the result of the victory of the tissues over the parasite, incomplete though it be, for the destruction of the bacilli is all but complete.[116] Some few bacilli remain and can be cultured from the tissue; hence the virulence of these tissues for susceptible animals and the conclusion that the bacilli are not destroyed in the immune animals. However, even in a disease such as vaccinia with its solid immunity, the virus has been concentrated by cataphoresis by Olitzky and P. H. Long,[117] long after immunity had been established.

This interpretation of the significance of the epithelioid cells and tubercles finds confirmation in the work of Thomas.[118] Sabin and her co-workers[119] in their studies on the role of the phosphatid of the tubercle bacillus in the genesis of the epithelioid cell support this interpretation for they found that as the lipid phagocyted by the monocytes undergoes finer and finer dispersion in their cytoplasm they assume the character of epithelioid cells. Since the liberation of the lipid from the tubercle bacillus and its elaboration by the phagocytes must occur chiefly after the death and disintegration of the bacilli, it is clear that epithelioid cell formation would be associated with the death of the bacilli.

Dienes and Mallory[120] have shown that normal guinea pigs respond with an exudation of polymorphonuclears to the introduction of tubercle bacilli; however, following tuberculous infection and synchronous with the development of hypersensitivity to tuberculin, reinjection of the micro-organism elicits a predominantly mononuclear reaction. Again, Lurie[121] found that the more rapid mobilization of mononuclear phagocytes by the tuberculous and relatively immune animal is an expression of the increased physiologic activity conferred upon these cells by the tuberculous process, for not only do the mononuclear phagocytes accumulate more rapidly in response to the tubercle bacillus but also non-specific irritants such as aleuronat or mineral oil elicit a more rapid mobilization of mononuclear phagocytes in the tuberculous than in the normal animal. This is associated with a demonstrable increase in the rate of amitotic and mitotic division which these cells undergo in the tuberculous as compared with that in the normal animal in their reaction to these non-specific irritants. Furthermore, both *in vivo* and *in vitro*, the mononuclear phagocytes derived from a tuberculous animal exhibit increased phagocytic activity not only for tubercle bacilli but also for unrelated particulate matter such as carbon and collodion particles. This increased physiologic activity of the mononuclear phagocytes of the tuberculous animal is a property of the cells themselves and is independent of the presence of normal or immune serum and, therefore, is not the result of the bacteriotropic action of the immune serum. This increased physiologic activity of the mononuclear phagocytes of the tuberculous, allergic and immune animal, which had previously been inferred, is now definitely demonstrated. This fact perhaps explains the increased capacity of tuberculous animals to form antibodies, as demonstrated by Lewis and Loomis,[122] and the intensification of pre-anaphylactic sensitization in tuberculous animals to antigens in general, as found by Dienes.[86] It is this increased physiologic activity of the mononuclear phagocytes of the tuberculous animal which is perhaps responsible for the increased proteolytic activity of the liver of immunized as compared with normal

rabbits.[123] This same enhancement of physiologic cell activity specifically oriented explains the greater destruction or inhibition of growth of tubercle bacilli in the mononuclears of immunized animals. Lurie[124] caused tubercle bacilli to be phagocytized *in vitro* by mononuclears derived from normal and immunized rabbits in the presence of normal or immune serum and carbon particles. The number of tubercle bacilli ingested by these cells was determined by culture. The normal cells with their load of bacilli and carbon particles were introduced into the anterior chamber of one eye of a normal albino rabbit. Into the other anterior chamber of the same rabbit were introduced the "immune" cells with their ingested tubercle bacilli and particles. After two weeks' growth in this identical *in vivo* environment the two cell types were removed and the number of bacilli they contained was again determined by culture. The cells were identified microscopically by their ingested carbon particles as the albino host had no pigment in his iris cells. It was found that active tuberculosis confers on the mononuclear phagocytes themselves increased bacteriostatic properties against the tubercle bacillus which are independent of the immune body fluids or of the organ environment in which they grow. Qualitative tissue culture studies by Kallos[125] confirmed these quantitative results.

The essential mechanism in immunity to tuberculosis is, therefore, an increased capacity of the mononuclear phagocytes to destroy tubercle bacilli. With small doses of reinfection in a highly immune animal the bacilli are immediately and completely destroyed[115] by the mononuclear phagocytes *in situ*, presumably leaving little if any lipid residue, and hence these cells do not even assume an epithelioid structure. Boquet[126] comes to the same conclusion.

It has been shown by Mudd and his coworkers[127] that promptly following reinfection there is a rise in agglutinating and phagocytosis-promoting antibodies in rabbits that are effectively destroying the bacilli of reinfection. Lurie[46] mixed tubercle bacilli with melted agar and injected the mixture subcutaneously into normal and immunized rabbits. The body fluids readily penetrate the agar which quickly solidifies *in vivo;* the cells invade the agar islands slowly. Under these conditions it has been found that the body fluids of normal animals support the growth of tubercle bacilli, whereas the body fluids of the immune animal that penetrate the acellular agar islands *in vivo* do not support their growth. Furthermore,[50] silk bags were impregnated with a collodion solution of such strength that it permitted the passage of body fluids but prevented the entrance of cells. When such bags containing virulent tubercle bacilli are placed in the peritoneal cavity of normal and immune animals, it can again be shown by culture that in the complete absence of cells the body fluids of the immune animal which penetrate these bags *in vivo* inhibit the growth of the bacilli as compared with the growth of the bacilli in bags placed in normal animals. This is supported by the observation of Thomas and Duran-Reynals[128] who have caused a marked reduction in the rate and extent of development of tuberculous skin lesions in rabbits by mixing the inoculum with an antipolysaccharide serum obtained from a horse treated with living tubercle bacilli.

Furthermore, by the use of the agar focus[46] it can be shown that as Rich[52] had found with the pneumococcus so it is with the tubercle bacillus. In the acellular agar islands of the normal animal the bacilli grow widely dispersed, whereas in the immune animal they are more often found in the form of minute dense clumps, indicating that the surface properties of the bacteria bathed in the immune body fluids tend to make them adhere to each other and reduce their mobility in the tissues. The more pronounced fibrin barrier deposited about the site of reinfection and the thrombosis of adjacent lymph vessels which occurs about the focus in a highly sensitized animal will also aid in fixation of the bacilli. However, even if these growth-inhibiting, immobilizing and fixing properties of the body fluids are

ineffective, the mononuclear phagocytes with their increased capacity to destroy tubercle bacilli are rapidly mobilized and are brought to the site of reinfection and to the invaded lymph nodes where they promptly destroy or effectively inhibit the multiplication of the bacilli of reinfection. It is noteworthy that the impotent polymorphonuclears disappear more rapidly from the site of reinfection than from the site of primary infection.

Thus by appropriate *in vivo* studies it can be shown that acquired resistance in tuberculosis is primarily a function of the cells, but the humoral substances of the immune animal also play a role. It is important to emphasize at this point that the suppression of acute inflammation by treatment with tuberculin does not eliminate the most significant factor in immunity to tuberculosis which is the increased capacity of the mononuclear phagocytes to destroy tubercle bacilli, as in the accelerated formation of tubercle. To the extent that the suppression of allergy diminishes the accumulation of these phagocytes, interferes with their acquired physiologic activities, reduces the thrombokinase released and retards the clotting process and the thrombosis of draining lymph vessels and lowers the concentration of humoral substances at the focus of reinfection, it may be said the reduction of allergy may lower the effectiveness of the immune process. However, the suppression of the acute inflammation, as effected in the reduction of the number of impotent granulocytes brought to this focus, may actually aid in the immune process for, as emphasized by Albert Weil,[129] they act chiefly as agents of dissemination of tubercle bacilli.

As to the essential nature of the change in the mononuclear phagocytes which increases their bactericidal and/or bacteriostatic properties on tubercle bacilli, little is known except that they may be a reflection of the increment of their non-specific physiologic properties, and among these their rate of division and their phagocytic and digestive capacities. As to what is responsible for the specific orientation of these properties to the destruction of tubercle bacilli, nothing is known. It is possible that specific antibodies may be involved which have an avidity for cellular attachment similar to those now being demonstrated as playing a role in tuberculin allergy but developing in response to different antigens such, perhaps, as the surface lipids of tubercle bacilli. Whatever these may be it must be emphasized that the rapidity and intensity of the acquisition of these powers by the phagocytes as a result of exposure to tubercle bacilli are largely determined by the genetic resistance of the host and hence are conditioned by the host's constitutional factors and are unrelated to the parasite. A slight immunization with BCG or even with heat-killed tubercle bacilli will completely protect a rabbit of high genetic resistance against a challenging infection while the same treatment of a susceptible rabbit will only prolong his life for a few months as compared with the survival of an untreated similarly infected littermate.

There is a strange fact that is at variance with all that has been said. Although the tuberculous animal readily destroys the bacilli of reinfection, even if these are highly virulent, the primary lesions may harbor innumerable bacilli even if these are of lower virulence. These primary lesions may gradually extend and kill the animal. Whether this results from an adaptation of the bacilli to the immune state of the animal[130] or to other circumstances is unknown.

It is the persistence of bacilli in old foci which makes the immunity in tuberculosis so variable. Although the bacilli are scanty and gradually tend to die in old caseous foci, separated as they are from the blood stream, during the process of softening which sets in with penetration of blood constituents and often air into the previously immured foci, the residual bacilli here undergo tremendous multiplication in the dead cellular debris. Living phagocytes which penetrate these softening foci die as a result of their allergic sensitivity. The

enormous numbers of living bacilli now spread through preformed channels such as the bronchi and renal tubules or by way of the blood and lymph and overwhelm the existing immunity. The mechanism of softening, which is the most important fact in the mortality of human tuberculosis, is unknown.

Preventive Vaccination against Tuberculosis. Living virulent tubercle bacilli have been cautiously given to man,[131] beginning with single bacilli and gradually increasing. The method had to be abandoned as too dangerous. BCG is a typical tubercle bacillus which multiplies in the body for a short time[114] but is soon destroyed. In the regional lymph nodes isolated organisms persist[132] for a ong time without causing macroscopic lesions or acquiring increased virulence. It produces typical tuberculous lesions, sometimes extensive, including caseation, but with the all important difference that the changes regress and disappear completely. It brings into play the factors tending to immobilize the bacilli of reinfection, inhibit their growth and destroy them with a resulting significant immunity. As long as the organism is propagated according to the directions of Calmette there is no danger of its becoming virulent. The various factors involved in the production of the most desirable vaccine and the mode of its dispensation are now being widely studied.

In the early period of its use, when the BCG was still in the process of attenuation, certain investigators succeeded by special cultural procedures or by animal inoculation in enhancing the growth of the small proportion of still virulent bacilli that were at that time contained in the culture. Since 1929 the attenuation has become so complete that in the last few years evidence has come forth[133] that there is a possibility of the strain becoming too attenuated and hence useless as a vaccinating agent. In any case there is no authentic evidence that in the 1,500,000 infants to whom it was given it has caused a single death. The disaster in Lübeck was apparently due to an error;[134] the same culture sent to Lübeck was innocuous in another laboratory.[135] The protection that it affords is only moderate but apparently sufficient to reduce definitely the tuberculosis mortality of exposed individuals,[136] as indicated by the few carefully controlled studies at hand. The degree of heightened resistance conferred by the BCG is superimposed and determined by the innate resistance of the individual vaccinated. Therefore, it would seem advisable to determine the efficacy of the most desirable preparation of the vaccine on at least two different strains of animals, one of high genetic resistance and one of very low resistance, for both types are present in human populations. Moreover, the increased resistance thus acquired by these two strains may be advantageously gauged by the method of natural quantitative inhalation[16] of single or repeated minimal doses of virulent tubercle bacilli for this simulates both the mode of inception of tuberculosis and the quantities of bacilli involved in natural human infection. The vaccine is best given parenterally as this insures penetration of the vaccine into the tissues and will be indicated by a positive Mantoux reaction. The duration of the immunity conferred is at least five years. It can scarcely be expected that it will protect against marked exogenous reinfection during adult life for even the virulent tubercle bacillus is ineffective under these conditions.

The possibilities of a killed vaccine for prophylactic use in man are still problematic. There is little agreement as to its efficacy in experimental animals. Recently, vaccination with dead tubercle bacilli in combination with other antigens[137] or irritants[138] has yielded promising results in animals. Its use in man has thus far been greatly limited. It is possible that an effective vaccine, derived from an organism of proper antigenic composition, and perhaps in combination with other agents, may still be elaborated.

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Pathogenetic Concepts of Tuberculosis*

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THE development of technics to stain and to grow mycobacteria has demonstrated that there are a considerable number of widely dispersed species of which only a few are pathogenic. Also, it is now known that several animal species other than man naturally acquire mycobacterial infections. Although the disease in different genera of animals usually is caused by different species of mycobacteria, a pattern of macroscopic and microscopic changes that presents a number of similar features often develops in affected organs. Frequently it is possible to make a diagnosis of tuberculous disease without identification of the specific etiologic agent. One of the common characteristics is the necrotic lesion. The theme of the discussion in this article will be to indicate the place that the necrotic lesion occupies in the pathogenesis of tuberculosis in man and the significance of the necrotic focus in relation to the clinical manifestations of the disease.

An examination of the tissues of a person who has died from tuberculosis reveals macroscopic lesions that vary widely in size, consistency and general appearance. Microscopic study of the variety of macroscopic lesions shows a considerable variation in histologic features, all of which are related basically to the dynamic phenomenon of inflammation and repair of injured tissue. A similar condition is found in the study of animals that have succumbed to naturally acquired tuberculous disease. Commonly these macroscopic and microscopic features are regarded as specific for tuberculous disease; however, it appears probable that whatever specificity there may be rests upon a chemical rather than a morphologic basis.

Necropsy findings in human tuberculosis may be likened to the witnessing of the final act of a drama with all the actors on stage. The recorded attempts to reconstruct the pathogenesis of the disease from the study of necropsy material are probably as accurate as a detailed reconstruction of a drama would be from the witnessing of the final act. Usually the histologic tubercle is selected as the initial morphologic response; the presence of acute inflammatory reaction commonly is interpreted either as an "allergic" phenomenon or as due to the presence of other bacteria, hence "nonspecific," and the processes that precede the phenomenon of necrosis, commonly called "caseation-necrosis" in tuberculosis, if described at all, are portrayed in vague terminology. Frankly, the initial morphologic response to the first implantation of tubercle bacilli in human tissue is not known and without knowledge of this phase in the pathogenesis a correct interpretation of the sequence of events in the human disease is not possible.

It is commonly assumed that man contracts pulmonary tuberculosis from the inhalation of minute air-borne particles which are contaminated with tubercle bacilli. The experimental production of pulmonary tuberculosis with air-borne bacilli by Wells[1] and Lurie[2] indicate that droplets or particles must be invisible and extremely small to reach and to become implanted in the pulmonary parenchyma. This appears to be reasonable for I have failed to find recorded in the literature a description of a prenecrotic tuberculous lesion of macroscopic proportions that could be interpreted as an early air-borne infection. In my

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search at necropsy at the Office of the Medical Examiner for the Borough of Manhattan for evidence of tuberculous infection in the tissues of over 1,000 persons who met sudden death, I failed to find a single prenecrotic tuberculous focus of macroscopic size that could be attributed to an air-borne infection, although macroscopic evidence of tuberculous infection was found in three-fourths of the bodies examined. Small, necrotic, tuberculous foci were observed occasionally as well as other evidence of this infection, ranging from fibrocalcific lesions which were 1 mm. or more in diameter to extensive pulmonary disease. During this study it was not uncommon to find macroscopic lesions of various dimensions consisting of acute, nontuberculous, lobular pneumonia. These observations serve to emphasize the difficulty of portraying clearly the pathogenesis of pulmonary tuberculosis from the study of human tissues only.

COMMENTS ON EXPERIMENTAL TUBERCULOSIS

The experimental approach to the pathogenesis of pulmonary tuberculosis is inviting. Caution is required, however, in any attempt to apply to man the results obtained experimentally in animals. This becomes a subject in comparative pathology wherein the complex chemical processes of life that control the differentiation into different orders in the animal kingdom have not been clarified. On a morphologic basis all vertebrates have comparable systems of organs that perform similar functions. Also, if living tissue is injured, the process of inflammation and repair is exercised universally to counteract the damage. It seems reasonable to regard all infectious diseases as a chemical process wherein living tissue is injured and to interpret the morphologic processes that are utilized to overcome this injury as the observable pathogenesis of the disease. The process is dynamic with a constant interplay of chemical and morphologic forces until the injury has been completely overcome or the host has perished.

In the process of inflammation and repair all vertebrates use similar morphologic tools, the various leukocytic types. Proportionally and numerically these leukocytic types vary from species to species and from individual to individual within a single species. In the general phenomenon of inflammation and repair each of the leukocytic types apparently perform a specific function that is analogous in all species of animals. It is the accumulation of these various morphologic units at the site of tissue injury in infectious diseases that creates the demonstrable macroscopic and microscopic lesions. Since the process is dynamic, the proportion of the different leukocytic types in a lesion may vary from time to time in response to changing chemical conditions. The sequence of changes related to the time of introduction of the injurious agent constitutes the pathogenesis of the lesion.

In morphologic studies of experimentally produced tuberculosis some factors may be chosen arbitrarily whereas others require investigation. Choice is optional of the species of host and parasite, the numbers of the parasite to be given, the way that the parasite is to be implanted in the tissues and the time interval after the parasite is introduced that sacrifice of the host for study will be made. Factors to be evaluated are the pathogenic or non-pathogenic effect of the parasite upon the host, the morphologic reaction to the tissue injury induced by the parasite and the time sequence of morphologic changes that follow the implantation of the parasite.

It can be demonstrated readily that all species of tubercle bacilli which cause a natural progressive disease do not affect equally all species of animals and that in a single species of animal the reactions to different species of pathogenic tubercle bacilli may vary widely. Differences in the reaction to a pathogenic and a non-pathogenic strain of the same genus of tubercle bacillus also can be demonstrated with ease. However, in a species that is of an order significantly different from the natural host great difficulty is encountered in attaining a con-

sistent reproduction of disease that is similar in all respects to the disease in the natural host.

Reiteration of some of my observations in the study of experimentally produced tuberculosis in animals will serve to illustrate the intricacies of this problem. Although progressive and consistently reproducible tuberculosis is obtained readily in the guinea pig with either human or bovine virulent bacilli, wholly necrotic lesions like those commonly seen in man or in cattle seldom are encountered. Tuberculous lesions in this animal behave more like abscesses observed in other infections in that although the tissue of an organ may necrose the inflammatory reaction, which consists mainly of neutrophiles, it shows much less evidence of necrosis. In the rabbit the results are unpredictable when virulent human type tubercle bacilli are used whereas with virulent bovine bacilli progressive disease, with the major feature being extensive involvement of the lungs, can be obtained consistently regardless of the way in which the bacilli are first introduced into the tissues. The necrotic pulmonary lesion that is so common in man is also a predominant feature in the rabbit although it is present in an infection caused by the bovine type of bacilli. With virulent avian tubercle bacilli guinea pigs are affected little whereas intravenous injection of these bacilli in the rabbit results in a progressive and reproducible disease in which the lungs are affected only slightly; the spleen, liver and bone marrow are affected extensively; the reaction consists almost wholly of monocytes and no macroscopic or microscopic evidence of necrosis that is analogous to the necrotic human lesion occurs although bacilli are present in large numbers in the lesions. Intravenous injection of large numbers of non-pathogenic bovine bacilli (BCG) in the rabbit is followed by a considerable inflammatory reaction, especially in the lungs, that consists almost wholly of monocytes and lymphocytes. The height of this reaction occurs in about a month after infection and complete resolution ensues within six months without any necrotic lesions in most instances. On rare occasions these bacilli may succeed in multiplying to considerable numbers in isolated foci and in such lesions the neutrophile is the predominant leukocytic type. With destruction of the bacilli in these lesions the necrotic lesion is produced, and in the repair of this necrotic lesion "epithelioid" tubercles, with or without giant cells, and calcification with fibrosis usually occur.

Intravenous injection of virulent bovine tubercle bacilli into rabbits previously vaccinated with non-pathogenic bovine bacilli (BCG) gives a pattern of disease in the lungs quite unlike that obtained in virgin soil. In primary infection with virulent bacilli lesions develop and progress equally in all parts of the lung parenchyma to death of the animal. In the reinfected animal, although lesions develop initially in all parts of the lung parenchyma, they do not progress uniformly. The foci in all except the dorsal and caudal portions of the lung parenchyma slowly regress and heal completely; in the dorsal and caudal areas large necrotic lesions develop and persist for many months. Frequently these large necrotic lesions slough and this leads to cavity formation and often to endobronchial dissemination of the disease. A pattern identical with that found in reinfected rabbits can be obtained in primary infection provided that a proper balance is obtained between the natural resistance of the normal rabbit and the virulence and numbers of bovine bacilli. This pattern could not be obtained in primary infections unless the infected animal lived more than a year and unless few bacilli of "moderate" virulence were used to initiate the infection. It is significant that an identical pattern of chronic pulmonary tuberculosis can be obtained in the rabbit in both primary and reinfection tuberculosis, for in some respects this experimentally produced disease resembles that commonly observed in man.

The pattern of blood-borne pulmonary tuberculosis in the rabbit with the large necrotic lesions in the higher portions of

lung parenchyma resembles closely the pattern found in cattle with naturally acquired air-borne disease. However, in cattle the lesions are usually unilateral, frequently are single in number and commonly there is considerable involvement of the lymph nodes in the line of drainage from the parenchymal focus. In these respects the experimentally produced disease differs. Since the location of large necrotic lesions in the rabbit under certain controlled conditions and in cattle is similar and since the location of similar necrotic pulmonary lesions in man is in the higher portions of pulmonary lobes, most often in the upper lobes, an attempt was made to influence the location of the progressive and necrotizing lesions in the rabbit by a forced change in posture from a horizontal to an upright position. The influence of posture on the well-being of the animal soon became evident. Without adequate abdominal support and adjustment of the length of time that the animal was kept in an upright position complications such as torsion of the gut with gangrene, chylous ascites or aspiration pneumonia occurred. Indeed, survival of normal animals was uncertain. After many changes in procedure a few tuberculous animals survived for four months and in such animals a shift of the progressive and necrotic lesion from the caudal to the cephalic portion of the lung parenchyma was obtained. The explanation of this phenomenon of selective location of progressive pulmonary lesions remains, for the present, speculative. However, it seems evident that the determining factors are not the way by which tubercle bacilli reach these areas, the species of pathogenic bacillus that is used or the question of a first or a subsequent infection.

From these observations it appears that the necrotic pulmonary lesion so commonly observed in the rabbit infected intravenously with virulent bovine tubercle bacilli is the counterpart of the necrotic lesion so commonly found in pulmonary tuberculosis in man. It also seems reasonable to assume that the morphologic processes that precede the phenomenon of necrosis is analogous. In the rabbit the bacilli are few in a lesion during the days immediately after inoculation and the inflammatory reaction consists of a mixture of monocytes and neutrophiles. Within two weeks the bacilli have multiplied to considerable numbers and by this time the neutrophile has become the predominant leukocytic type in a majority of the lesions. This condition persists for another week or two with further multiplication of bacilli and with continued accumulation of neutrophiles without macroscopic and microscopic evidence of extensive necrosis of the lesions. At this stage there is some microscopic evidence of ulceration of lesions into bronchi with initiation of endobronchial dissemination of the disease. Beginning about the fourth week, progressive and extensive necrosis of the whole lesion occurs which affects the inflammatory reaction and the lung tissue alike, and before long bacilli become much less numerous in the lesions. In animals that survive for three months or longer it is often difficult to demonstrate bacilli in the older necrotic foci whereas they are numerous in the more recently disseminated lesions. It seems that in the development of the progressive tuberculous lesion there is a similarity to abscess formation in other infectious diseases preceding the initiation of the destruction of tubercle bacilli. Also, necrosis of all elements accompanies the destruction of considerable numbers of tubercle bacilli. The reason for failure to accomplish prompt liquefaction and slough of this necrotic lesion in the rabbit remains to be elucidated. However, it seems plausible that the enzyme systems which seem to be related in some manner to the neutrophiles may be greatly impaired by the process of coagulation necrosis. Also, it would seem to be a reasonable assumption that the processes which precede the appearance of the phenomenon of necrosis in the rabbit likewise occur in man.

In my studies of experimentally produced pulmonary tuberculosis in animals I was unable to duplicate in all respects the morphologic conditions known to exist in

man. However, I believe identical individual lesions have been obtained; necrotic pulmonary lesions in both rabbit and man are composed in large part of dead neutrophiles and lung parenchyma; in both rabbit and man the necrotic lesion is most difficult either to resorb, to organize or to slough; with the sloughing of the necrotic debris, ulceration with eventual cavity formation and endobronchial dissemination occurs; concomitant with the sloughing process the tubercle bacilli remaining within the necrotic area commonly find an environment conducive to renewed and rapid growth; the histologic tubercle with its giant cells and minute focus of necrosis represents a reparative process that is of greater significance as a diagnostic than as a menacing feature of the disease.

COMMENTS ON PULMONARY TUBERCULOSIS IN MAN

In any consideration of human pulmonary tuberculosis its microscopic beginning should always be emphasized. In a study of minimal pulmonary tuberculosis[3] I have pointed out that although the disease may appear to be early in a clinical sense, it is not so to be considered in a pathologic sense. The extent of the disease does not indicate necessarily the age of the lesion. Necrotic tuberculous lesions that have sloughed sufficiently to give rise to local endobronchial dissemination seem to precede any roentgenologic evidence of the presence of a lesion. On a pathologic basis the major difference between minimal and advanced disease, as seen in roentgenograms, concerns mainly the volume of lung parenchyma affected. Under these circumstances the significant pathologic facts in a case of pulmonary tuberculosis are the following: an area of necrotic tuberculous pneumonia is present and it has preceded the date at which roentgenographic shadows are first observable; the necrotic lesion has sloughed sufficiently to permit endobronchial dissemination; the sloughed area need not be large enough to make possible a demonstration of cavity formation and the roentgenographic shadows represent both prenecrotic and necrotic lesions that cast similar shadows.

One of the variables present in pulmonary tuberculosis is the quantity and the bacillary content of sputum. Persons with roentgenologic evidence of cavity formation usually, although not always, discharge sputum in which bacilli can be demonstrated in smear preparations. Some patients show little roentgenologic evidence of disease, are not aware that they raise any sputum and yet may yield positive cultures repeatedly from fasting gastric contents. In others with roentgenographic shadows of considerable extent repeated cultures and animal inoculations may fail to reveal bacilli. On occasion an increase of pulmonary disease may occur without the discharge, at the time or subsequently, of bacilli in demonstrable numbers. From a comparison of preoperative smear examinations and cultures with the demonstrable bacillary content of lesions in resected lung specimens, it is my experience that failure to demonstrate bacilli in sputa or fasting gastric contents is no guarantee of a scarcity of bacilli in necrotic lesions with patent communications with bronchi.

The presence of tubercle bacilli in sputum usually if not always indicates the presence of a sloughing parenchymal lesion whereas their absence does not indicate the absence of such a focus. There is no evidence that tubercle bacilli *per se* can induce the liquefaction of necrotic tissue elements. However, there is evidence that necrotic tissue, whether induced by an infectious or a non-infectious process, can be liquefied through the chemical forces of the body and there is some morphologic evidence which suggests that this is probably true in the liquefaction of necrotic tuberculous foci. The sloughing of a necrotic tuberculous lesion may and often does occur at a time when bacilli are present in large numbers. Also, sloughing may occur at a time when the bacillary content either is low or even non-existent, although the latter condition probably is unusual.

Tubercle bacilli are not found with equal facility in all lesions that represent the various phases of pathogenesis. They seldom are found and then only in small numbers in "epithelioid" tubercles, and they can be demonstrated even less often in Langhan's giant cells. They may or may not be numerous in lesions of recent origin in which the neutrophile may be the predominant cellular component. It is my belief that in many pulmonary lesions in tuberculosis the inflammatory cellular response is a reaction to the irritating effect of the inhaled necrotic material *per se* rather than to the few bacilli which may be present. Frequently the bronchus which drains a sloughing lesion shows congestion, edema and considerable lymphocytic infiltration of the mucosa without evidence of implantation of tuberculous infection. It is my belief that this condition is caused more by the irritating effect of the debris as it oozes along the bronchus than by its bacillary content.

The numbers and distribution of bacilli within necrotic lesions vary widely. Neither the macroscopic nor the histologic appearance can be used as an index of their location. In lesions that present a similar morphologic appearance one lesion may reveal no bacilli in sections from different areas; in a second every section may contain an occasional bacillus; a third may show large numbers of bacilli within a small area in only one of the sections and in a fourth lesion bacilli in considerable numbers may be present and may be distributed fairly uniformly in all sections. Usually the bacilli are located deep within the necrotic portion rather than in the border which is adjacent to the encircling fibrotic wall and frequently they are present in greatest numbers in necrotic areas which are adjacent to the sloughing part of a lesion. At times large numbers of bacilli in colony formation may be found deep within a necrotic lesion without any evidence of migration of leukocytes toward the area. Similar conditions exist relative to the bacterial content of tuberculous cavities although bacilli usually are much more numerous. It is rare, indeed, to find an open tuberculous cavity which fails to reveal the presence of bacilli in microscopic sections.

There is no current procedure, short of actual examination of lesions, which permits accurate evaluation of the bacillary content of tuberculous foci. The bacilli present in sputum represent in large part and in a rough way the bacterial population of actively sloughing lesions. Under a variety of conditions, one of which may be the temporary plugging of a bronchus with thick viscid debris, the numbers of bacilli may increase or decrease. Such fluctuations do not indicate necessarily that either a favorable or an unfavorable change in the lesions has occurred, and a cessation of discharge of bacilli need not indicate that the tuberculous process has become sterile. The persistence of large numbers of bacilli in the discharge from a lesion certainly is a great hazard to the infected individual for the fate of a new area of disease depends in large measure upon the numbers of bacilli present at its inception. However, a new pneumonic focus heavily seeded with tubercle bacilli may occur in a patient who is unaware of the presence of sputum as well as in one who raises a considerable amount of sputum which contains large numbers of bacilli. Because of these varying conditions it would appear that great emphasis should not be given to a "conversion" of sputum as an indication of permanent improvement in the disease process. One of the unexplained phenomena in tuberculosis is the frequent survival of a few bacilli in an environment which destroys large numbers of these organisms and the subsequent prolific growth of these survivors within the necrotic debris. This phenomenon, combined with long-delayed sloughing of a necrotic lesion, may well be the decisive factor concerned in the reappearance of bacilli in sputa after they have been absent for a long time.

The danger from the necrotic tuberculous pneumonic lesion resides in the fact that it is extremely difficult to resorb, to organize or to slough and that living tubercle bacilli, in variable numbers, can persist within it

for a long and indefinite period. In time these necrotic lesions present a peripheral zone of fibrosis that often is interpreted as creating an innocuous closed lesion. It is essential to recognize that previous to necrosis the lesion was a lobular pneumonia with a patent connecting bronchus and that the condition of this bronchus may be of greater importance than the fibrotic periphery so easily observed. With necrotic lesions that are less than 2 cm. in diameter macroscopic recognition of the connecting bronchi is most difficult because of their minute size. Such a lesion may be compared to an inflated rubber balloon with the small uninflated stem representing the bronchus. Unless serial microscopic sections of the balloon-like necrotic lesion be made, an onerous task that seldom is performed, the condition of the bronchus proximal to the necrotic area can hardly be determined. However, if many random sections from many necrotic lesions are studied microscopically, a bronchus may be found on occasion which lies peripheral to the fibrotic encapsulation and which exhibits lymphocytic infiltration of the submucosa, with or without the presence of Langhan's giant cells and "epithelioid" tubercles and with the presence of necrotic debris within the lumen. Fortuitously one may find the fibrotic "capsule" interrupted by the presence of a bronchus in tangential section which in the inner portion communicates directly with necrotic debris and in the outer portion presents a fairly normal structure. Rarely is any evidence found which indicates that a bronchus has become obliterated by scar formation. These findings indicate that more often than not the bronchus to the necrotic pneumonic focus is patulous although it may be plugged by necrotic debris. Within the necrotic area a framework of dead alveolar walls, vessels and bronchi frequently can be demonstrated and a slough cannot occur without disintegration of this framework. At times liquefaction with slow oozing through the connecting bronchus may be delayed so long that focal deposition of salts (calcification) has occurred in the peripheral portion. It is the open bronchial communication with the apparently walled-off necrotic pneumonic lesion together with the potential, although unpredictable, liquefaction of the area that causes pulmonary tuberculosis to be characteristically a chronic relapsing disease.

Usually the necrotic portion of a lesion does not slough completely and the space vacated by the slough (cavity formation) is lined by a varying amount of necrotic debris in which tubercle bacilli, still present although perhaps in small numbers, find an environment favorable for growth. In an effort to rid the body of the remaining necrotic remnants, leukocytes, predominantly neutrophiles, invade the area evidently to induce liquefaction of the debris. Peripheral to the necrotic area granulation tissue, which commonly has an abundant blood supply, is laid down as a reparative measure. Apparently considerable numbers of tubercle bacilli are destroyed in this process for commonly they are found distributed unevenly with the largest numbers present near the free surfaces of necrotic debris not invaded by neutrophiles. It seems that this condition induces a vicious cycle which causes necrosis of the granulation tissues as considerable numbers but not all of the bacilli are destroyed, and that repetitions of this process probably are responsible for the common failure of complete repair of cavity formation by fibrosis.

Clinical experience indicates that closure of cavity is a desirable procedure and on pathologic grounds this is sound practice for this usually creates an environment less favorable for continued rapid growth of bacilli. However, the mechanical closure of a cavity approximates two necrotic surfaces that cannot knit together readily since the necrotic debris is most difficult to resorb or to organize. Often the necrotic material becomes inspissated and thus the lumen of the connecting bronchus may become plugged without the production of a barrier of scar tissue across it. Under this condition drainage from the area may be stopped so long as liquefaction is not again induced.

It is this situation that makes possible the reopening of cavities subsequent to closure under any therapeutic regimen.

Dissemination of tuberculous infection within the lung parenchyma from a sloughing pneumonia area is only one manifestation of the necrotizing process. Breakdown of necrotic, non-thrombosed blood vessels may lead either to hemorrhage or to widespread hematogenous metastases. Drainage of the necrotic debris through the airways may induce implantation of the disease in these structures and swallowing of the purulent drainage may be followed by implantation of bacilli in the intestinal tract. Sloughing of necrotic lymph nodes into bronchi may cause extensive endobronchial dissemination, and erosion into vascular channels may give rise to generalized metastatic foci. Metastatic lesions in other organs often undergo necrosis and softening that may bring about extensive involvement of such organs. It seems evident that the major underlying pathologic feature of all clinical manifestations of this disease is the phenomenon of necrosis which precedes the unpredictable and hazardous process of liquefaction and slough.

No tuberculous lesion is necrotic in its early inflammatory (exudative) stage; all inflammatory lesions do not undergo necrosis and inflammatory lesions may exist for a long time without undergoing necrosis. This situation creates great difficulty in the interpretation of pulmonary roentgenographic shadows for there is no means of differentiation between non-necrotic and necrotic foci. Neither is it possible to differentiate, by the character of roentgenographic shadows, between necrotic foci, non-necrotic inflammatory foci of long duration and scar formation. Serial roentgenograms serve primarily to indicate either progression, regression or lack of demonstrable change in the disease process. The factor of greatest significance in roentgenographic shadows of recent origin in tuberculosis is the possibility of necrosis, with subsequent slough and the development of new areas for dissemination of the disease. In roentgenographic shadows of long-standing lesions the factor of greatest danger is the frequency, the persistence and the potentialities for sloughing of necrotic areas of tuberculous pneumonia.

Although wide variation in the size and in the extent of sloughing of necrotic lesions can be demonstrated, the underlying process is the same in all.

The following cases will serve to illustrate the significance of the necrotic lesion:

A white male at the age of thirty-five years was found to have a dense, well delineated roentgenographic shadow that measured 3 cm. across in the left upper lung field. No change in his clinical status occurred for seven years at which time a less dense and less well defined roentgenographic shadow of approximately the same dimensions was discovered in the right upper lung. At this time sanatorium care was advised and accepted. During the next three years of conservative hospital care only minor roentgenographic changes were observed on the right side; however, tomography finally revealed a highlight that was interpreted as a cavity formation. With stability of the roentgenographic shadows for over a year, the demonstration of small numbers of tubercle bacilli occasionally on cultures of sputa, the presence of cavity formation and the excellent physical condition of the patient, excision of the right upper lobe seemed justifiable.

Examination of the excised lobe revealed no cavity formation. However, there were two separate foci of disease with normal lung parenchyma between them and it appeared that this situation was responsible for the highlight that was interpreted as cavity formation. The smaller of the two foci measured 1 by 1 by 2 cm. and lay toward the medial surface of the lobe. On section this lesion was firm throughout, was of a yellowish gray color and macroscopically appeared to be well encapsulated. The larger lesion lay toward the lateral surface of the lobe and measured 2 by 2 by 4 cm. On section this lesion was solid and presented a macroscopic appearance similar to the smaller lesion. Both lesions lay in the upper portion of the posterior segment. Otherwise the pulmonary tissue presented a normal macroscopic appearance.

Microscopic study showed a zone of fibrosis peripheral to the two large necrotic lesions with cholesterol crystals and small focal areas of salt

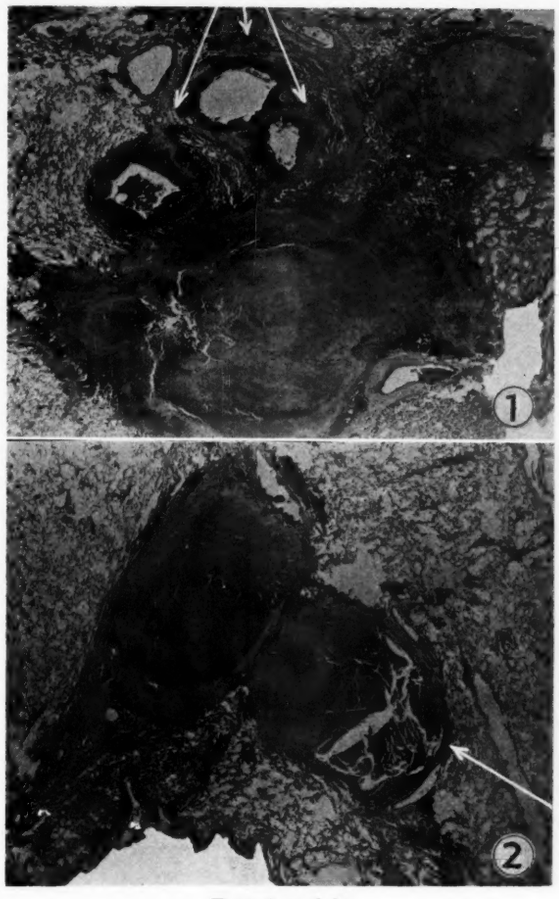
deposit in the necrotic debris that was adjacent to the fibrotic "capsule." Patulous bronchi were found to connect directly with the necrotic part of the two large lesions and in this part of the lesion there was abundant exudation of easily identified neutrophiles. An exudate composed largely of neutrophiles was present within the connecting bronchi, and the bronchial mucosa exhibited a considerable degree of lymphocytic infiltration with the presence of an occasional monocytic (epithelioid) tubercle. Tubercle bacilli were present in small numbers in the viable purulent exudate and deep within the necrotic debris of the larger lesion bacilli were present in large numbers in some areas. This latter finding is of particular significance for it indicates that the paucity of bacilli in the sputum did not reflect the true bacillary content of the large necrotic lesions.

The true status of these necrotic foci would not have been determined had not numerous sections of the lesions been examined microscopically. In particular, the lesions might have been regarded as closed if a careful examination of their bronchial connection had not been made. Another important feature of the microscopic study was the finding of minute foci of tuberculous pneumonia which showed a considerable variation in microscopic appearance. This indicated that they varied from a rather old to a recent origin. In other words, microscopic endobronchial dissemination was inducing the formation of new lesions within a roentgenographic shadow that presented a picture of stability for over a year. The presence of bacilli in the exudate within the bronchi suggests that further dissemination of the disease probably would have occurred.

This patient still has the lesion in the left lung field which remains unchanged. It probably represents a necrotic tuberculous pneumonic lesion and presumably was the source from which the endobronchial dissemination to the right side arose. Its future course is unpredictable and requires watching. It is well that the right upper lobe was excised; however, this procedure has not solved the problem completely.

In Figures 1 and 2 the bronchi which were proven to communicate directly with the two large necrotic lesions are indicated by arrows.

The situation found in this case is not infrequent. Figures 3 to 6 are from four other resected lung segments or parts of segments in which bronchi, indicated by arrows, were found

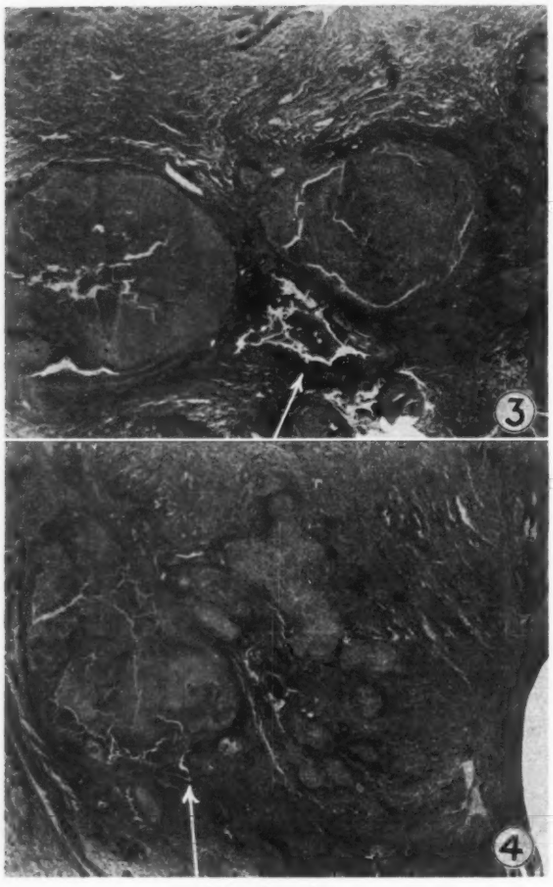


FIGS. 1 and 2.

to communicate openly with necrotic pneumonic lesions.

Figure 3 is from a block of lung tissue resected at the time decortication of a lung was done. The lung failed to re-expand after effective pneumothorax had been maintained for two years. No growth of tubercle bacilli had been obtained from culture of several specimens of sputum and of gastric contents after the induction of pneumothorax. Several necrotic foci were present in the resected specimen with the largest being 1.5 cm. in diameter. Bacilli were numerous in some of the necrotic lesions.

Figure 4 is from a segment of lung tissue removed under conditions similar to the previous case. This disease has been known to be present for about five years in the portion of lung field which was removed. Tubercle bacilli had been demonstrated repeatedly; however, for six months prior to surgery no bacilli were demonstrated by culture of sputum. There were several necrotic lesions present in the specimen with the largest measuring 2 cm. in cross section.

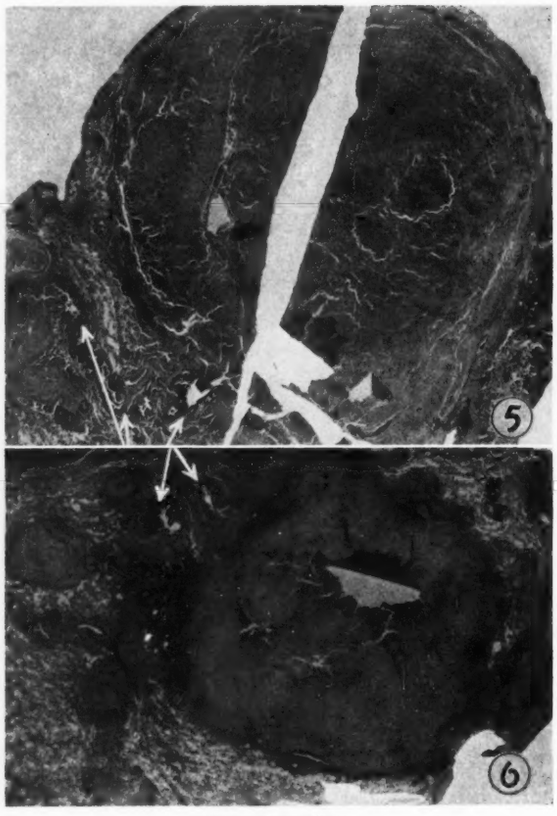


FIGS. 3 and 4.

Prolonged search was required to find any bacilli in the tissue sections.

The lesion illustrated in Figure 5 was known to have been present for over a year and on serial roentgenograms it appeared as a dense, well delineated, almost spherical shadow. From repeated cultures prior to surgery only one gave growth of a few colonies of tubercle bacilli. The major portion of the resected block of lung tissue was occupied by a solid necrotic lesion which measured 3 cm. in cross section. Tubercle bacilli were numerous deep within the necrotic area. No collapse measures or chemotherapy had been used prior to surgery.

Figure 6 is from a lesion which had been observed over a period of five years. The only change in the roentgenographic shadow during this period was the appearance of cavity formation about nine months prior to surgery. Only two of several cultures of sputum gave growth of small numbers of bacilli. No collapse measures or chemotherapy was used prior to surgery. The major portion of the block of lung tissue which was removed was occupied by a



FIGS. 5 and 6.

necrotic lesion which measured 3 by 5 cm. on cross section. An area 2 by 3 cm. in cross section in the central portion of the lesion had been sloughed. Tubercle bacilli were numerous and were irregularly distributed in the necrotic area. They were most abundant in the zone bordering the cavity formation.

It appears that necrotic areas of tuberculous pneumonia commonly are in communication with patulous bronchi and that the bacillary content of such necrotic foci frequently cannot be determined by culture or other methods of examinations of sputum or of fasting gastric contents. Also, it seems evident that the sloughing of necrotic pneumonic lesions is not determined by the quantity of tubercle bacilli within the lesion.

Sufficient clinical experience has now been gained to demonstrate that certain clinical manifestations of tuberculosis can be markedly affected by appropriate chemotherapeutic agents, and on a pathogenetic basis the lesions that respond to these agents are in the prenecrotic state. Lesions in this condition may be either of short or of long

duration and the only way to differentiate them from the necrotic lesion is by the manner in which they respond to the application of chemotherapeutic agents. From a study of necropsy and surgical specimens there is no evidence at present that the necrotic lesion is affected in an appreciable morphologic manner by any agent used so far other than perhaps a reduction in the numbers of bacilli in such foci. It is essential that the complete pathologic problem inherent in tuberculosis be given greater consideration than is commonly the case. It is gratifying to obtain with chemotherapeutic agents subsidence of fever, marked improvement in the sense of well-being in a patient and considerable melting away of roentgenographic shadows. However, these immediate responses should be interpreted with proper perspective of the problem as a whole, which necessarily must place great stress upon the persistence and dangers of necrotic lesions that antedated the acute clinical and roentgenologic manifestations of the disease. It is evident that chemotherapy favorably affects the prenecrotic phase of the pathogenesis of tuberculosis more rapidly than previous orthodox methods of treatment. It also appears probable that pneumonic foci that have not proceeded to necrosis may be prevented from so doing through the bacteriostatic effect of chemotherapeutic agents. However, the pathologic problem that still remains to be solved is the recalcitrant necrotic, pneumonic tuberculous lesion.

This discussion has been limited intentionally to a presentation of the morphologic features of tuberculous infection. These features have been considered as evidence of a reaction to chemical injuries to tissue; and since the underlying chemical phenomena are unknown, the tuberculous lesions have been viewed as an exhibition of the general, non-specific, dynamic phenomenon of inflammation and repair. The phenomenon of allergy, of which hypersensitivity to tuberculin (or tuberculoprotein) is one manifestation, has not been included in this discussion because it is my belief that such phenomena are concerned with chemical rather than with morphologic processes. Also, error is unavoidable if an attempt is made to interpret specific chemical reactions on a morphologic basis. One morphologic evidence of the influence of allergy in tuberculosis is a more rapid response and a larger volume of cellular exudate at the site of tissue injury in the allergic host. However, this is more a quantitative than a qualitative response. The morphologic response to tuberculin injected into a sensitized host proceeds through the various phases that are found in the universal process of inflammation and repair, and it seems illogical to designate any one phase of this dynamic process as evidence of hypersensitivity. It is my belief that the same logic should be applied to all of the morphologic manifestations of tuberculous disease. This does not deny that on a chemical basis both allergy and hypersensitivity may exert a profound effect on the disease. It does indicate that whatever the effect may be it should be defined in specific chemical rather than in morphologic reactions.

SUMMARY

Accurate portrayal of the pathogenesis of tuberculosis from the study of human tissues alone is not possible. To reproduce experimentally tuberculosis that duplicates in all respects the disease as seen in man is extremely difficult, but in the experimental animal it is possible to obtain individual lesions that morphologically are identical with similar lesions in man. Also, it is possible in the experimental animal to determine the morphologic sequence of events occurring in the development of the different lesions. In man it is evident that the phenomenon of necrosis with subsequent liquefaction and sloughing of the necrotic areas represents the major problem in the dissemination and in the complete eradication of the disease in the person affected. In the experimental animal it can be shown that the lesion which undergoes necrosis is composed in large part of neutrophiles and contains large numbers of tubercle bacilli

prior to the occurrence of necrosis. Concomitant with the destruction of tubercle bacilli in considerable numbers the entire lesion, both the inflammatory exudate and the formed tissue structure, necroses until so-called "caseation necrosis" is complete. The process resembles an abscess commonly seen in other infections up to the stage at which all morphologic elements are killed. The ultimate disposal of the necrotic area presents the major problem, for neither resolution, organization nor sloughing is readily brought about. Sloughing results in ulceration which, if large, gives rise to demonstrable cavity formation by roentgenograms. Sloughing of a necrotic focus creates a condition more favorable for the growth of tubercle bacilli that may have survived the process inducing necrosis of the lesion.

Further destruction of bacilli in the inner portions of the cavity apparently causes further necrosis of exudate and of granulation tissue. In this manner a vicious cycle is established which greatly hinders complete repair of cavities. There is extreme variability in the number of bacilli in necrotic lesions. Also the bacillary content of sputum frequently fails to indicate the bacillary content of necrotic lesions.

It is essential to recognize that some sloughing of a necrotic lesion antedates the development of the clinical manifestations of tuberculosis, even in disease of minimal extent. In addition, the necrotic lesion with its potentiality for sloughing remains after marked clinical improvement, whether this has followed a regimen of bed rest or of chemotherapy. Furthermore, it is my belief that the problem posed by the necrotic lesion includes biochemical phenomena which induce lysis of tubercle bacilli in considerable numbers and, therefore, requires investigations along lines other than study of the bacteriostatic or bactericidal effect of chemotherapeutic agents.

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Course and Prognosis of Tuberculosis in Children*

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THE pattern of tuberculous infection and disease is the same in children and adults. In the adult, however, the onset of tuberculosis is rarely clinically manifest and signs of early generalization may not be evident. In the child the stages of tuberculosis are often distinct and well separated. In children rather than in adults one is privileged to study what Wallgren[1] has aptly termed the "time-table of tuberculosis." Every form of tuberculosis may be seen on a children's ward and it is possible to follow the evolution of the disease from first infection to phthisis, although the pediatrician sees cases mainly of primary tuberculosis with its immediate sequelae and relatively few cases of chronic pulmonary tuberculosis. The clinical course of chronic pulmonary tuberculosis is essentially the same in children and in adults. The following discussion of the course of tuberculosis in children will concern itself largely with the manifestations of primary and post-primary tuberculosis.

No classification of tuberculous disease has been devised that is perfect, but Ranke's[2] division into three stages furnishes a helpful basis for the interpretation of clinical observations. He postulated a first stage of the primary complex in which the spread of the disease is by way of the lymphatics; a second stage of generalization of the infection in which the predominant spread is by the blood stream. In the third stage of Ranke's classification the disease is again localized to one or more principal areas. This is the stage of chronic isolated phthisis in which the predominant lesion may be in the lung (the so-called reinfection type of tuberculosis) or in other parts of the body.

PATHOGENESIS

The portal of entry of the infection is probably more important in determining the subsequent course of tuberculosis than the type of infecting bacillus. Since bovine bacilli are usually ingested in milk, the intestinal tract is the usual point of entry, consequently mesenteric adenitis and peritonitis are frequently seen as part of the picture of primary tuberculosis due to infected milk. The human type of bacillus is usually inhaled but may also be ingested. If the portal of entry is in the intestinal tract, the clinical pictures produced may be identical with those formerly described as peculiar to tuberculosis of bovine origin.

Tuberculosis is usually caused by direct infection from an individual with active tuberculous disease and in the great majority of cases the bacilli are conveyed by droplet infection through a cough or sneeze and are inhaled into the lungs by the recipient. The first infection lesion is usually just under the pleura and the bacilli soon create a small area of tuberculosis which is known as the primary focus. Almost as soon as infection takes place bacilli begin to leave the primary focus and are carried through the lymphatics to the nearest group of lymph nodes draining the area in which the primary focus lies.[3] This combination of the

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primary focus, a corresponding lesion in the regional nodes and the interfocal zone joining these two areas of tuberculosis is known as the primary complex. In the early days of infection the body tissues have not been sensitized to tuberculin and consequently during the incubation period of the disease the Mantoux or other tuberculin skin reactions will be found to be negative. After tuberculous infection has become established a change occurs in the reaction of the tissues to the tubercle bacillus and its metabolic products. With the appearance of allergy a perifocal infiltration may develop around the primary tuberculous focus, the regional nodes become larger and both components of the primary complex may become visible on x-ray. A perifocal reaction around a tuberculous focus resembles in its morphology any exudative tuberculous tissue reaction.[4] The size and intensity of the reaction depend largely on the extent of the tuberculous disease at the portal of entry and especially on the number of bacilli and the amount of tuberculo-toxins which are present. The size of the perifocal reaction may also vary to a lesser extent with individual factors such as race and age and with other factors about which little is known. Perifocal reactions around primary foci tend to clear slowly and even a small lesion usually remains visible on x-ray for at least three months. It is not unusual to see larger shadows persist for several years.

In the great majority of cases the perifocal reaction around the parenchymal lesion gradually subsides and the primary focus at its center heals with fibrosis and calcification. This benign behavior of the lesion at the portal of entry has unfortunately led many clinicians to the conclusion that primary tuberculosis is itself a benign disease. Less commonly the parenchymal primary lesion may caseate, excavate and progress by bronchogenic spread. If the destruction is extensive, healing is probably often incomplete and the lesion may constitute a menace to the future health of the child. In the cases seen at Bellevue Hospital this form of progressive primary tuberculosis is more frequent in Negro than in white children.

The caseous lesions in the regional nodes may be small if the infection is light. But even when there is little local progression of tuberculosis in the nodes, the tendency to complete healing is not as great as in the parenchymal focus. Caseation and living tubercle bacilli often persist in nodes for long periods of time even when partial calcification has taken place. If the infection is heavy, an entire group of nodes may become caseous. Not infrequently other nodes beyond the regional nodes become involved and ultimately caseous tuberculosis may develop in the tracheobronchial nodes. The tendency for massive spread beyond the regional nodes is most marked in very young children and the tuberculous nodes also tend to be larger in the infant than in the older child.

Because of the anatomic relation of the paratracheal nodes to the lymphatic duct and blood stream, the possibility of progression of primary pulmonary tuberculosis by the lymphohematogenous route is always present. From clinical and pathologic evidence it is probable that at least a few bacilli reach the blood stream in most if not all tuberculous infections during childhood.[2,3]

Obviously not every tubercle bacillus which enters the blood stream results in an anatomic tubercle. Undoubtedly there are constitutional and racial or inherited variations in immunity and there is a known difference in organ susceptibility. The thyroid, pancreas and stomach are rarely involved but this is only a relative immunity since tuberculous disease may occur in these organs. Pathologic or roentgen evidence of seeding during the postprimary lymphohematogenous spread is most frequently found in the spleen and the apices of the lungs. The development of tuberculous disease in the metastatic area depends not only on local and general immunity but largely on the dosage of bacilli implanted in any one area and on the rhythm of infection; prolonged or repeated seeding produces more progressive metastases than a

single dissemination. Any tuberculous seeding may develop directly into an active tuberculous complication; or it may regress and heal completely; or it may remain quiescent but containing living tubercle bacilli. Many years after first-infection tuberculosis such a latent focus may develop into an area of active tuberculosis.

The familiar picture of acute generalized miliary tuberculosis is the result of a single hematogenous dissemination of a large number of bacilli. This condition usually results from the invasion of a blood vessel by a caseating focus of tuberculosis or by dissemination from a tubercle within the lumen of a blood vessel originally caused by a tubercle bacillus seeded during the post-primary lymphohematogenous spread.[5] If generalization occurs, the resulting clinical and pathologic pictures will depend largely on three factors: the topographical location of the disseminating focus, the dosage and the frequency of seeding of tubercle bacilli. All clinical pictures of acute and chronic hematogenous tuberculosis can be explained by variations in these factors.

CLINICAL PICTURE OF TUBERCULOSIS AT PORTAL OF ENTRY

Symptoms of Invasion and Onset. With an understanding of the pathogenesis of tuberculosis recognition and interpretation of clinical tuberculosis in the child should not be difficult. The incubation period of tuberculosis is the time between the first inhalation of tubercle bacilli and the development of an altered tissue reaction to the tubercle bacillus and its metabolic products. It can be measured by the tuberculin reaction which usually becomes positive in three to five weeks after exposure, with a probable range of two to seven weeks.[6] Knowledge of the mode of onset of tuberculosis has been gained by studying children who have been exposed to a tuberculous individual and who are known not to have been previously infected. Through observation of such cases it has been found that during the incubation period the x-ray, tuberculin test and clinical picture remain negative. (Fig. 1.) The onset of the disease is heralded by a fever rarely over 102°F. and lasting approximately three to ten days. Usually, except for the anorexia or lassitude which may accompany the fever, there are no other symptoms or physical signs. If the

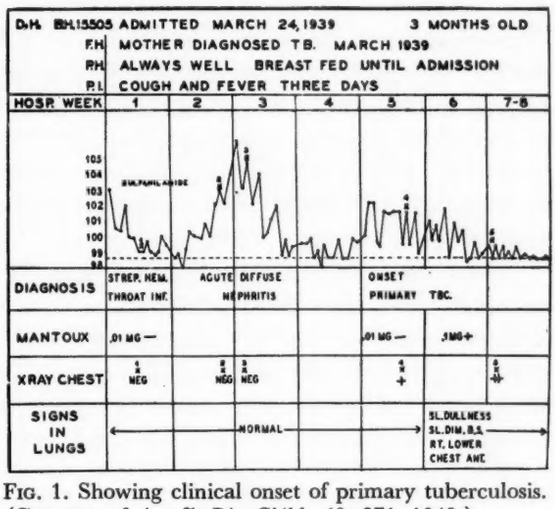

FIG. 1. Showing clinical onset of primary tuberculosis. (Courtesy of *Am. J. Dis. Child.*, 60: 371, 1940.)

pulmonary and nodal components of the primary complex are to be visualized by x-ray, they are usually seen on the first x-ray taken after onset. The parenchymal shadow then remains unchanged during the succeeding weeks or months; rarely does it increase in extent during the first days or weeks of the disease. With massive infection the size of the nodes may ultimately increase. The tuberculin reaction usually becomes positive with the first appearance of fever. Occasionally the appearance of a positive tuberculin reaction is delayed.[7]

Because of the insidious nature of the beginnings of tuberculosis it is easy to see why most cases are not diagnosed in the earliest stage. Sometimes the onset of tuberculosis is more dramatic and simulates the clinical picture of lobar pneumonia with physical signs of a consolidated pulmonary area, including rales, rapid respirations and the appearance of an acutely ill child. The absence of pneumococci in the sputum, the character of the x-ray with enlarged nodes as well as a parenchymal lesion and the presence of a positive tuberculin reaction may reveal the true diagnosis. Often the

tuberculous nature of the disease is not discovered until it is found that the x-ray picture remains unchanged after the fever and symptoms have subsided. Many cases of primary tuberculosis with this type of onset are not diagnosed promptly because an x-ray is not repeated after the child has apparently recovered. It is possible that the occurrence of the pneumonic type of onset is determined by the degree of tissue change or allergy. Long follow-up studies of children with both types of onset have revealed no difference in ultimate prognosis or rate of clearing.

As far as is known the clinical picture of onset of first infection tuberculosis is the same whether or not the pulmonary lesion ever becomes visible on x-ray. In cities like New York where more than 99 per cent of primary infections are found in the lung it is reasonable to suppose that when the x-ray picture remains negative, in a child with a recent conversion of the tuberculin test, that the primary lesion is well hidden or too small to be seen. A recent conversion of the tuberculin test therefore should mean active pulmonary tuberculous disease in any child and the x-ray helps only in determining the degree and location of the infection. It is well known that the very young child has little immunity to tuberculosis. It is not surprising therefore that in the Children's Chest Clinic at Bellevue Hospital more than 80 per cent of infants with positive tuberculin tests had roentgen evidence of primary tuberculosis. The probability of finding a primary complex on x-ray following conversion of the tuberculin test decreases with age. Beaven[8] has studied the relation of tuberculous infection to clinical disease in various age groups and concludes that in the age group 0–14 tuberculous infection results in clinical evidence of tuberculous disease much more frequently than in adults.

Clinical Picture of Primary Pulmonary Tuberculosis. There is a striking lack of physical signs and symptoms during the early postprimary stage of tuberculosis. Even with extensive parenchymal exudate there is little or no cough and tubercle bacilli can be demonstrated only by gastric lavage and not in every case. Fever may be absent after the initial rise or there may be an irregular low grade fever with occasional rises to 101°F. Very infrequently one sees the association of a hectic type of fever with large masses of caseous nodes but this febrile response is often absent in the presence of massive disease of the nodes. Persistent high fever early in the disease often precedes tuberculous meningitis or miliary tuberculosis. The most constant symptom of primary pulmonary tuberculosis elicited from the parents is a tendency for the child to become fatigued more easily than before the onset of the disease. Abnormal signs in the lungs are rarely found even in the presence of a large x-ray shadow. At most, slight dullness or diminished breath sounds are heard and the absence of rales is so striking that the presence of even a few constant moist rales should lead one to suspect locally progressive disease and bronchogenic spread or non-tuberculous pulmonary infection.

Symptoms and signs due to encroachment of enlarged nodes on bronchi are characteristic. The most common symptom is a harsh, repeated expiratory cough and this may become so severe that it becomes brassy in character. If the enlargement of the encroaching nodes is the result of perifocal reaction due to tuberculinization or intercurrent disease, the symptoms may be transient. Usually the nodes are caseous and the condition persists for weeks or months. Sometimes there is actual compression of a bronchus by a large mass of nodes; more often the nodes become adherent to the bronchus due to inflammatory changes. As the disease progresses endobronchial ulceration is frequently produced and this can be demonstrated by bronchoscopy. Wheezing respirations are often present at this stage and rhonchi are frequently heard. A mistaken diagnosis of asthma or pertussis is not uncommon. The x-ray picture often confirms the diagnosis of obstruction by showing a homogeneous clouding of a segment of a lobe or occasionally an entire lobe. As endobronchial disease progresses polypoid

caseous masses may be formed causing both obstruction and emphysema.

Sudden cessation of the signs and symptoms of obstruction, especially when accompanied by a change to a loose cough, should suggest evacuation of the contents of a node through an ulcerated area in the bronchial wall. The resulting clinical picture will depend on the physical character of the material evacuated and on the number of tubercle bacilli present. There may be no clinical sequelae of a minimal bronchogenic spread; a larger amount of caseous material and tubercle bacilli may result in caseous pneumonia. Tuberculous endobronchitis not infrequently ends in spontaneous cure but may result in deformity or permanent obstruction of a bronchus.

If the child develops intercurrent respiratory infection while the bronchus is obstructed, the potential danger of development of bronchiectasis is always present. Suppurative pulmonary disease is not an infrequent complication of first infection tuberculosis in children but rarely interferes with healing of the tuberculous process. Symptoms due to encroachment of tuberculous nodes on the intestinal tract may also occur, causing distention and partial obstruction and in rare cases perforation.

CLINICAL PICTURES OF OCCULT HEMATOGENOUS DISSEMINATION

Progression of primary tuberculous disease by the hematogenous route may not cause symptoms or signs. But frequently within ten weeks of the onset of the disease evidence of blood stream invasion may be found. The spleen often becomes easily palpable and sometimes definitely enlarged and a general enlargement of all superficial lymph nodes usually takes place. A few papulonecrotic tuberculides may appear and possibly one or two phlyctenules on the conjunctiva. This picture of what has been termed "occult hematogenous tuberculosis"[9] usually lasts only a few days to a week. It may have no clinical sequelae. In 2 to 3 per cent of the cases followed at Bellevue Hospital apical calcification, so-called "Simon foci"[10] were later seen on x-ray as a result of postprimary lymphohematogenous seeding. These apical foci may vary in size from miliary lesions to larger irregular calcifications. During this postprimary period other manifestations of seeding through the blood stream may take place. Red blood cells may be found in the urine, due to the formation of a few tubercles in the pelvis of the kidney; tubercle bacilli may be cultured from the urine even when urinalysis is normal. Sometimes during this period children may exhibit physical signs and symptoms suggestive of meningitis and go on to complete recovery. This clinical picture of serous tuberculous meningitis[11] is not uncommonly seen on a ward for children with active tuberculosis and is probably due to a perifocal inflammation around tubercles seeded into the cortex during the postprimary period of dissemination or to later reactions around such foci. Differential diagnosis from caseous tuberculous meningitis is important because most cases of serous meningitis resolve spontaneously and do not require chemotherapy. In the first stages of caseous tuberculous meningitis the differentiation from serous meningitis cannot be made by the clinical picture since the same signs and symptoms may appear in either. Examination of the spinal fluid is of more help. While an increase in spinal fluid pressure and in number of cells is found in both forms, no one has reported changes in the sugar, protein or chloride content of the spinal fluid in serous meningitis.

EARLY COMPLICATIONS OF POSTPRIMARY DISSEMINATION

Following the period of postprimary lymphohematogenous spread, frank metastases may develop from progression of individual seedings anywhere in the body. Tuberculosis of the skeletal system is a frequent complication occurring most commonly as spondylitis; dactylitis is the form of bone tuberculosis usually seen in the infant. Tuberculous involvement may occur in any group of superficial lymph nodes but most commonly in the cervical region.

Tuberculous otitis media is characterized by lack of pain and recurrent discharge usually unaccompanied by fever. Epididymitis is a less frequent metastatic complication. Small areas of mottling of military size may appear on x-ray. Meningitis is the most dreaded early complication of primary tuberculosis, usually occurring within the first six months after infection is established. From the evidence of Rich and McCordock[12] and others meningitis is due to the invasion of the subarachnoid space through extension of a previously established caseous focus in the cortex, meninges or adjacent bone structures. Since meningitis is now amenable to specific therapy, its early recognition is important. Unfortunately the usual method of onset is insidious and neurologic examination is often entirely normal during the first stage of general symptoms. Sudden onset of persistent fever or rise from a low grade to higher degrees of temperature should always arouse suspicion of meningitis in a child known to be tuberculous, especially in the first months of the disease.

Early diagnosis of meningitis in children not known to be tuberculous will be accomplished if routine Mantoux tests are made in children with fever or general symptoms of unknown origin which do not yield to the usual methods of therapy. In the series of cases of tuberculous meningitis studied on the Children's Medical Service of Bellevue Hospital positive tuberculin reactions were obtained in 85 per cent of the children and roentgen evidence of manifest primary tuberculosis was present in over 95 per cent.[13] The importance of early diagnosis is emphasized by the finding in the Bellevue Hospital series that children with untreated tuberculous meningitis lived only an average of nineteen and one-half days.

GENERALIZED HEMATOGENOUS TUBERCULOSIS

The complications of tuberculosis just discussed all develop from foci of caseous tuberculosis distributed originally by way of the blood stream or lymphatic system. If the secondary focus happens to be within the lumen of a blood vessel or if a caseating focus invades the blood stream, a large number of bacilli may be distributed at one time. Acute generalized tuberculosis will result and the chest x-ray alone will make the differential diagnosis between the uniform size lesions of miliary tuberculosis and those of larger size. In all acute generalized forms enlargement of spleen and superficial lymph nodes may be present but are often absent. In the more chronic forms of generalized hematogenous tuberculosis enlargement of nodes and spleen are more constantly found. Fever is usually present at the onset of acute hematogenous tuberculosis and is unaccompanied by other symptoms except irritability. Like meningitis, acute generalized hematogenous tuberculosis usually manifests itself in the first six months after the development of first infection tuberculosis.

When a caseous focus invades the blood stream and causes repeated seedings of varying doses of tubercle bacilli, a striking clinical picture of hematogenous tuberculosis results. In this form, termed protracted multiform hematogenous tuberculosis,[14] there is usually massive involvement of all the superficial lymph nodes and a panserositis resulting, in severe cases, in caseation of serous surfaces. The spleen is often enormously enlarged, there may be associated tuberculosis of the skeletal system and the x-ray shows pulmonary nodules symmetrically distributed but of unequal size. As in other forms of generalized hematogenous tuberculosis the multiform type may be acute, with death occurring within a few months of the onset of symptoms or chronic and lasting for years.

CASE FINDING

If the tuberculous nature of the disease is missed at the time of onset, it is often impossible to diagnose first infection tuberculosis in its early stages by ordinary clinical methods, through a recital of symptoms or by examination of the patient. The greatest aids in case finding are a

history of contact with a case of tuberculosis, the tuberculin test and chest x-ray.

A history of contact with an adult who has open tuberculosis usually results in the finding of a large percentage of positive tuberculin tests in children who have been exposed. Even when the tuberculosis in the adult is arrested, the percentage of child contacts reacting to a tuberculin test is higher than in families in which no case of tuberculosis exists.[15]

A positive tuberculin test indicates only that tuberculous infection has taken place and that living tubercle bacilli are present in the individual tested. Variations in the size and intensity of the reaction offer no help in evaluating the severity of the infection nor do they assist in determining the location or site of the anatomic lesion. A positive reaction is usually found in a tuberculous child unless he is suffering from a very acute and overwhelming form of the disease or is moribund; or unless the test is made within three weeks of the onset of measles. Occasionally high fever suppresses a positive reaction. Positive tests should not be repeated because they very rarely become negative during the span of childhood even if the primary complex is apparently well calcified, and because the injection of even small amounts of tuberculin in a positive reactor may cause a perifocal reaction around a focus of tuberculosis.[4] Intracutaneous (Mantoux) tests are the most reliable, using Purified Protein Derivative (P.P.D.), or old tuberculin (O.T.) in dilutions of 1:10,000, 1:1000 and, as a final test, 1:100. The use of P.P.D. eliminates many of the atypical reactions seen with O.T. On the other hand O.T. solutions possess the advantage of being more stable and remaining potent for at least a month. The tuberculin patch test is a useful adjunct to tuberculin testing chiefly because it is easy to apply and less disturbing to a child. Its chief value is as a preliminary screening test and for periodic retesting of previous negative reactors as a routine procedure or in contact cases. False positive reactions may occur and a physician should familiarize himself with the appearance of a positive patch test before attempting to interpret it.

An x-ray of all children with positive reactions is essential and serial films are of great help in diagnosis and prognosis.

With the exception of the relatively few cases discovered at the onset of the disease most diagnoses of first infection tuberculosis are made through tuberculin tests or x-rays obtained as a routine measure or because of a history of contact. In the early post-primary period the tuberculous child does not exhibit a characteristic facies but maintains his usual appearance. If he is well nourished and happy, his looks remain unchanged. Later in the disease and especially when there is extensive bronchogenic spread or intestinal tuberculosis, one may see the pallor and listlessness, the long eye-lashes and the general increase in body hair so often described as characteristic of tuberculosis. But by the time these signs appear the diagnosis of tuberculosis can be made easily by symptoms and signs.

ESTIMATION OF AGE OF TUBERCULOUS INFECTION

Unless one sees the child from the onset of tuberculosis it is impossible to estimate accurately the duration of the infection when established disease is found by means of tuberculin test or x-ray. However, in many cases an approximation of the age of the disease can be reached by study of the x-ray and clinical picture and by a knowledge of the time in the course of the disease when various complications are most likely to occur. Erythema nodosum is usually a very early manifestation of tuberculosis but unfortunately for the purpose of timing, it occurs only rarely in the United States.[16] Occult hematogenous tuberculosis is often the first evidence that dissemination has actually taken place; miliary tuberculosis and meningitis occurring in a child with a manifest primary tuberculosis are as a rule early complications. In a study of a large group of cases of tuberculous meningitis seen on the Children's Medical Service of Bellevue Hospital it was found that 43 per

cent of those who died of this complication did so within three months of the first diagnosis of tuberculosis. Primary pleurisy caused by a direct extension of the primary infection to the pleura may occur early but extensive pleurisy with effusion secondary

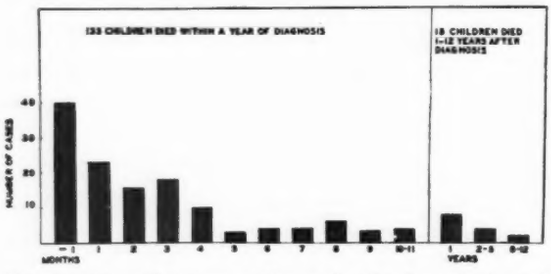

FIG. 2. Interval between diagnosis of tuberculosis and death; showing relation of mortality to duration of primary tuberculosis.

to hematogenous spread occurs later, usually after the tuberculous disease has been established for six to twelve months. Evidences of calcification in parenchymal or node components of the primary complex also help to measure the duration of the disease. The appearance of shadows of calcium density on the x-ray film is not uncommon in the infant as early as six months after onset of tuberculosis; as the child grows older calcification is not usually seen for at least a year and often not for two years or longer.

PROGNOSIS OF PRIMARY TUBERCULOSIS

The prognosis of primary tuberculosis in children depends on many factors and therefore statistics will always vary in different countries and in different sections of one country or city. The prognosis will vary to some extent with the social and economic status of the patient, with differences in diet and housing, possibly with racial differences and with other factors of which we know relatively little. During the past twenty years 622 children with manifest primary pulmonary tuberculosis have been followed in the Children's Chest Clinic of Bellevue Hospital in order to determine the prognosis of primary tuberculosis in this group. Over 85 per cent of the children were originally diagnosed as tuberculous through case-finding methods, usually through a history of contact or by a tuberculin test often done as a routine measure. The children came mainly from poor, overcrowded homes. Approximately half of the group were white, about one-quarter Negro, the remainder mostly Puerto Rican with a few Chinese. The case fatality rate in this group was high: 23.6 per cent died as a direct result of primary tuberculosis.

On further analysis the prognosis in these cases seems to depend mainly on three factors: The age of the tuberculous lesion, the age of the child and to a lesser degree the extent of the lesion. The interval between the diagnosis of tuberculosis and death is shown in Figure 2; 90 per cent of the deaths occurred within a year of the first discovery of primary tuberculosis, 74 per cent within six months and 58 per cent within three months. Therefore if the child survives primary tuberculosis for a year, he is unlikely to die of his disease. The age of the child when primary pulmonary tuberculosis is first diagnosed is also very important in the estimation of prognosis. Of infants first diagnosed when under six months of age 55 per cent died of tuberculosis; only 28 per cent of the older infants one to two years old died of primary tuberculosis and the mortality rate of the group four to nine years old was 15 per cent. The influence of the size of the primary complex is harder to determine but an attempt was made to measure the lesions on x-ray films and relate the results to prognosis. Forty-four per cent of those with the largest lesions died while only 23 per cent of those with minimal lesions had a fatal outcome. Further corroboration of the importance of the size of the lesion in relation to prognosis was obtained by following an additional group of infants under two years of age who showed only a positive tuberculin reaction with a negative x-ray. Of this group only 10 per cent succumbed to tuberculosis whereas more than 32 per cent of infants of the same age, with primary pulmonary tuberculosis visible on x-ray, died of the disease. The age of the patient was also of importance in relation

to the direct cause of death, as may be seen in Figure 3. The incidence of meningitis is lowest in the age group under six months when the case fatality rate is highest. Meningitis caused 60 per cent of all deaths from primary tuberculosis and in almost two-thirds of the cases acute generalized miliary tuberculosis was associated with the meningitis. Less than 10 per cent of the deaths were due to miliary tuberculosis without meningitis. Protracted hematogenous tuberculosis and locally progressive primary tuberculosis accounted in about equal proportions for 25 per cent of the deaths. Thus it will be seen that these four forms of tuberculosis were responsible for 95 per cent of the mortality from primary pulmonary tuberculosis.

The object of the prognosis study was not only to learn the immediate mortality rate of primary tuberculosis but to evaluate the potential dangers of the disease to those who recovered, to learn how frequently late complications occurred and if possible what factors influence their appearance. A third and very important objective of the study was to determine the number of children who would develop so-called "reinfect" pulmonary tuberculosis during adolescence or early adult life.

Late complications of primary tuberculosis are probably due mostly to progression of disease from metastatic foci laid down during postprimary seeding or occasionally from later seedings from a caseous focus. They present the same clinical pictures in children and adults and involve chiefly the skeletal system, lymph nodes, intestinal tract and pleura; less commonly other serous surfaces or the genitourinary tract. Frequently there is a history of trauma or of some other factor precipitating the development of a late complication. Few clinicians question the pathogenesis of the kind of late complications of primary tuberculosis which have been mentioned. In discussing the development of chronic pulmonary tuberculosis there is a wide difference of opinion as to whether the pulmonary disease like the tuberculous spondylitis or pleuritis originated from a progressive primary tuberculosis or is due to fresh exogenous infection.

In order to have a large group of children with primary pulmonary tuberculosis for the prognosis study 1,000 consecutive cases were

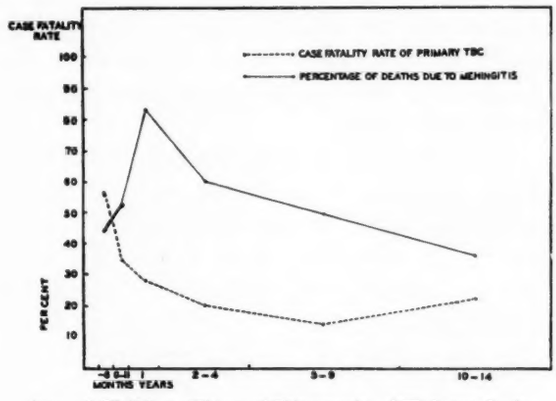

FIG. 3. Illustrates decreasing case fatality rate with age and variation in incidence of meningitis as a cause of death.

gathered in the Chest Clinic of the Children's Medical Service at Bellevue Hospital, beginning on October 1, 1930. These include the 622 children with recent primary tuberculosis which were used for the study of immediate prognosis; and in addition 378 children with roentgen evidence of partial or complete calcification of the primary complex. The survivors of this entire group are being followed to the age of 25 years, and more than 90 per cent have remained under supervision.

Eight per cent have developed pulmonary tuberculosis of the reinfection type one to fourteen years after primary tuberculosis was first diagnosed. Although the ratio of boys to girls was about equal in the original group, more than twice as many girls as boys later developed pulmonary infiltrates. The close approximation of the appearance of a pulmonary lesion on x-ray to the onset of menses was often striking. In more than one quarter of our cases a lesion of the reinfect type occurred within a year of the onset of menarche. Some relationship between adolescence in the female and the development of pulmonary tuberculosis is

suggested by these findings. In a small number of girls pulmonary lesions were first found between the ages of seven and nine years but the largest number of cases were found in the age group thirteen to fifteen years old. In only one patient could a renewed source of contact with active tuberculosis be demonstrated. These facts would seem to offer some support for the endogenous nature of pulmonary reinfection in this group.

Since the advent of chemotherapy it is no longer possible to study the natural course and prognosis of primary tuberculous infection. It is therefore more important than ever to continue to analyze available information on large groups of children collected before the days of streptomycin.

SUMMARY

The pathogenesis of tuberculosis can be readily studied in children because the various stages can be identified as clinical pictures. Primary pulmonary tuberculosis is always a disease of potentially serious import, as illustrated by the cases studied in the Children's Chest Clinic of Bellevue Hospital. The prognosis in this group has been greatly improved by chemotherapy of the complications previously responsible for the high mortality rate. By this method of selection of cases, treating less than 25 per cent of the ward population, it has been possible to reduce the case fatality rate for the past three and one-half years to 2.9 per cent. An understanding of the pathogenesis of tuberculosis, of the clinical pictures produced by the disease and of the prognosis of untreated cases remains of great importance not only in the proper selection of cases but in evaluation of the results of therapy.

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Effects of Antimicrobial Agents on the Tubercle Bacillus and on Experimental Tuberculosis*

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EVER since Robert Koch's momentous discovery of the tubercle bacillus investigators in the field of tuberculosis research have worked diligently to find an antimicrobial agent that was active in the test tube and in the animal body against this microorganism, and that was of sufficiently low toxicity to be well tolerated for relatively long periods of time.

In studying the effects of antimicrobial substances upon the tubercle bacillus the experimentalist must not only be familiar with their action upon the growth of the organisms in the test tube but also with their influence on different types of tuberculous infection in the experimental animal in order to apply the experimental findings intelligently to the treatment of the infection and the underlying pathologic lesions produced by the tubercle bacillus in man.

A complete review of the literature on the evaluation of the various agents which have been tested up to the present time would add little to this discussion except for its historical interest. Several such reviews have been published recently.[1–4] We shall confine ourselves primarily to the results obtained in the test tube and to animal experiments performed in this laboratory relating to those antibiotic and chemotherapeutic substances which have shown some promise in this field. The methods of *in vitro* testing of new agents and preliminary *in vivo* evaluation by short term animal experiments will be presented, followed by a comparison of the relative potencies of the more important antituberculosis agents and a discussion of each of these drugs individually.

TUBERCULOSTATIC EFFECT IN VITRO

The first step in the evaluation of an agent for its antituberculous activity is the testing of its growth-inhibiting power against the tubercle bacillus in the test tube. Saprophytic acid-fast bacteria may be used for preliminary screening, but in the final analysis virulent microorganisms should be used. The different types of media used and the common methods of testing are as follows:

Liquid Medium. The tubercle bacillus may be grown in liquid media either as a surface pellicle or submerged. The former type of growth, although satisfactory for carrying stock cultures and for the maintenance of virulence, is cumbersome and does not lend itself very well to accurate measurement. Submerged growth may be of two types, (1) in the form of clumps or aggregates; this is the manner in which tubercle bacilli tend to grow in liquid media unless a wetting agent is added; (2) in the form of diffuse turbidity; as accomplished in the liquid medium of Dubos[5] by the addition of the wetting agent Tween 80 and bovine albumin. The latter type of growth may be easily quantitated either by eye or by the exact measurement of the turbidity photoelectrically.[6]

* From the Trudeau Laboratory of The Trudeau Foundation for the Clinical and Experimental Study of Pulmonary Disease, Trudeau, N. Y. These studies were aided in part by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

Solid Medium. Drugs may also be incorporated into solid media. In some cases, such as with PAS, a sharp end point of inhibition may be obtained in such a manner when it cannot be obtained in liquid media.

The procedure in this laboratory is to use three types of liquid media for the initial trials, namely, the Tween-albumin medium of Dubos; Proskauer and Beck synthetic medium; and the latter medium with 10 per cent beef serum. The former medium is preferred because of the ease of handling and the convenience of its smooth turbid growth. It has been pointed out,[7] however, that the presence of the wetting agent, Tween 80, in this medium, may influence the bacteriostatic effect of certain drugs. There are some agents whose action is enhanced by it and there are some which are inhibited by it. The Proskauer and Beck medium is a simple synthetic medium without any interfering substances. Thus any influence of the wetting agent in the Tween-albumin medium will be brought out in the different behavior of the drug in these two media. The action of many agents is inhibited by the presence of serum. Comparison of the tests in the plain Proskauer and Beck medium with those in the Proskauer and Beck medium plus serum will show any effect serum may have on the antibacterial substance.

The culture used to supply the test organisms for these *in vitro* trials is usually the H37Rv (rough virulent) strain of human tubercle bacilli. In addition, the H37Rv strain resistant to streptomycin is subjected to the action of the antibacterial agent. Naturally, a new agent which is active against the streptomycin-resistant organisms offers more promise than one which is so like streptomycin as to be inactive against this strain.

In Table 1 are recorded the data obtained from the testing of actual or potential tuberculostatic agents. These tests (except when indicated) were all done in this laboratory using the technic described. Results are given for the fourteen-day incubation period. Test tubes 18 by 150 mm. were utilized, each tube containing 5 ml. of media. The size of the inoculum pipetted into each tube was 0.1 ml., containing approximately 0.03 mg., dry weight, or about ten million tubercle bacilli. The H37Rv culture was prepared for inoculation by growing it in Tween-albumin liquid medium for seven to ten days, at which time shaking produced a smooth turbid suspension of the microorganisms.

It will be noted (Table 1) that the concentration of some substances is recorded in mc. gm. per ml., of others in units per ml. It is unfortunate that all cannot be expressed on a weight basis; but since many of the antibiotic agents are relatively impure, they cannot be thus compared with pure chemical substances or crystalline antibiotics. The concentrations of the group comprising streptomycin, dihydrostreptomycin, streptothricin, SVI and neomycin are all based on the original Escherichia coli streptothricin unit as described by Waksman.[8] It was later found that 1 unit of streptomycin was equivalent to 1 mc. gm. of streptomycin base. Others, like terramycin and viomycin, are expressed in terms of weight of a relatively pure standard preparation.

Of the antibiotics, neomycin and streptomycin exhibit the strongest bacteriostatic activity against tubercle bacilli in the test tube. TB-1 and PAS* have activity of approximately the same order of magnitude as these antibiotic agents. It will be seen later, however, that this degree of *in vitro* bacteriostatic potency cannot be correlated directly with *in vivo* activity.

There are many other factors which should be investigated in regard to the *in vitro* action. For instance, does the drug have bactericidal effect as well as bacteriostatic effect? In other words, can the drug actually kill a certain percentage of the bacterial population in the culture or does it merely inhibit their growth so that when the organisms are withdrawn from the influence of this agent they may again multiply? It has been shown[9,10] that streptomycin and the sulfones have definite bactericidal powers, although even in very high concentrations streptomycin does not kill all of the individuals in a given culture of tubercle bacilli after prolonged contact.

* Abbreviations for 4-acetylaminobenzaldehyde thiosemicarbazone and para-aminosalicylic acid, respectively.

It is also shown (Table 1) that many of these drugs are rendered much less potent in the presence of serum. Such agents are in general much less active in the animal body than they are in simple synthetic culture media, as might be expected.

amount of tuberculous disease in the treated and control groups. The guinea pig was formerly used almost exclusively as the animal of choice in experimental tuberculosis, but the mouse has found increasing usefulness,[11–14] especially since Dubos and

TABLE 1

IN VITRO TUBERCULOSTATIC EFFECT OF ANTIMICROBIAL AGENTS IN THREE TYPES OF LIQUID MEDIUM IN APPROXIMATE ORDER OR ACTIVITY

Agent	Nature of Material	Minimal Inhibiting Concentration for H37Rv in mc. gm. or units/ml.			Action vs. SM-Resistant H37Rv
		Tween-albumin	P and B	P and B + Serum	
Streptomycin	Antibiotic	0.4 mc. gm.	0.4 mc. gm.	0.5–1.0 mc. gm.	Inactive
DHSM[1]	Antibiotic	0.4 mc. gm.	0.4 mc. gm.	0.5–1.0 mc. gm.	Inactive
DHSM-PAS[2]	Antibiotic and chemical compound	0.25 mc. gm.	.....	.....	Partial
Neomycin	Antibiotic	0.3 units	0.2 units	0.5 units	Active
TB-1[3]	Chemical compound	1 mc. gm.	1 mc. gm.	1 mc. gm.	Active
PAS[4]	Chemical compound	1 mc. gm.	1 mc. gm.	1 mc. gm.	Active
Viomycin	Antibiotic	5 mc. gm.	5 mc. gm.	5 mc. gm.	Active
Terramycin	Antibiotic	5 mc. gm.	5 mc. gm.	20–100 mc. gm.	Active
S VI[5]	Antibiotic	5 units	5 units	10 units	Active
Streptothricin	Antibiotic	5–10 units	.....	.....	Partial
O-aminophenol	Chemical compound	10 mc. gm.	10 mc. gm.	10 mc. gm.	Active
Aureomycin	Antibiotic	10 mc. gm.	10 mc. gm.	40 mc. gm.	Active
Chloromycetin	Antibiotic	20 mc. gm.	40 mc. gm.	40 mc. gm.	Active
Bacitracin	Antibiotic	10 units	100 units	> 100 units	Active
Subtilin	Antibiotic	1–5 units	> 100 units	> 100 units	Active
DDS[6]	Chemical compound	25 mc. gm.	.....	.....	Active
Promin[7]	Chemical compound	200 mc. gm.	200 mc. gm.	.....	Active
Aerosporin	Antibiotic	100 mc. gm.	1,000 mc. gm.	> 1,000 mc. gm.	Active

[1] dihydrostreptomycin
[2] dihydrostreptomycin-para-aminosalicylate
[3] 4-acetylaminobenzaldehyde thiosemicarbazone
[4] p-aminosalicylic acid
[5] streptothricin VI
[6] diaminodiphenyl sulfone
[7] results obtained by Smith[59] and Sasano[10]

TUBERCULOSTATIC EFFECT IN VIVO

The second step is to test the tuberculostatic effect of the drug upon an early, acute infection in guinea pigs and/or mice. The purpose of this preliminary investigation is mainly to determine whether the drug has any power to retard the infection, and for this purpose it is the usual practice to give a large infecting dose, to begin administration of the drug immediately and to kill the animals at an early date to compare the

his co-workers pointed out some of the factors involved in its susceptibility and resistance to tuberculosis.[15,16]

Mice offer some advantages for the preliminary *in vivo* screening, namely, only a small amount of drug may be available and mice require but little; laboratories with limited space for housing animals will find it a convenient animal; the expense of buying and maintaining mice is less than that of larger animals and the experiments can usually be terminated after four weeks. Also, it is not infrequently found that the

guinea pig is unable to tolerate a drug which is relatively non-toxic for other animals and for man. Penicillin and terramycin are examples of such drugs. In such cases one may still secure an evaluation using mice. We would not, however, depend on the mouse test alone but would

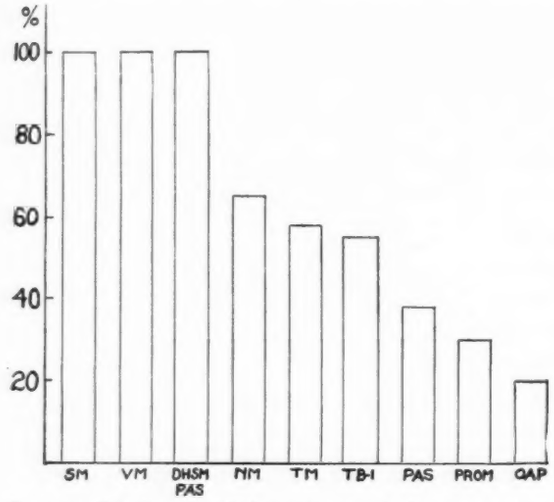

FIG. 1. Relative antituberculous activity of various antibacterial agents in guinea pigs as compared with streptomycin. SM, streptomycin; VM, viomycin; DHSM PAS, dihydrostreptomycin para-aminosalicylate; NM, neomycin; TM, terramycin; TB-1, 4-acetylaminobenzaldehyde thiosemicarbazone; PAS, p-aminosalicylic acid; PROM, promin; OAP, o-aminophenol.

prefer to confirm the results of such experiments in guinea pigs or rabbits, in which the gross and microscopic pathologic condition is more clearly defined and the intradermal tuberculin sensitivity may be followed from time to time.

In the standard preliminary test used in this laboratory guinea pigs are infected subcutaneously in the inguinal region with 0.1 mg. (dry weight) of the H37Rv strain of human tubercle bacilli. About two weeks later, when the animals will have developed skin hypersensitivity to 5 per cent old tuberculin, therapy with the drug is begun. The preferred method of administration is intramuscularly, but insoluble drugs may be given by mouth either in suspension or in capsules. Preliminary work is undertaken to determine the chronic toxicity of the drug for the guinea pigs, and the maximum tolerated dosage is administered for a period of forty-two to sixty days. At the end of this time all surviving animals are sacrificed. In these short term experiments relatively few animals die of tuberculosis, and any beneficial effect of the drug is revealed by a definite difference in the amount of disease between the untreated and treated groups.

A comparison of various potential antituberculous agents tested in this manner at the Trudeau Laboratory is presented. In Figure 1 the antituberculous activity of streptomycin is considered to be 100 per cent, and the activity of the other agents is compared with it. These materials were not all tested at the same time, but in practically all the experiments a group of animals was treated with streptomycin for comparison. We have not as yet seen any agent which exceeded the antituberculous potency of streptomycin, although viomycin and dihydrostreptomycin para-aminosalicylate produced beneficial results equal to it. Neomycin and terramycin showed about 65 per cent and 58 per cent, respectively, of the activity of streptomycin. TB-1 was a little more than half as active while oral PAS, in our hands, produced only 38 per cent of the effect of streptomycin. Promin and diaminodiphenylsulfone were about one-quarter as potent.

The following drugs were found to be devoid of significant *in vivo* antituberculous activity: aureomycin, subtilin, streptothricin, S VI and penicillin.

IN VITRO VS. IN VIVO ACTION

It may be seen from a comparison of Table 1 and Figure 1 that antituberculous activity in the experimental animal does not necessarily parallel *in vitro* activity. The reasons for this discrepancy may be the following: (1) Absorption of the drug may be so poor that adequate blood levels and tissue levels cannot be maintained. Too rapid excretion of the drug may produce the same results. (2) The toxicity of the drug may be so high that dosage adequate to produce an effect in the animal body cannot be given. (3) The drug may be either rapidly destroyed or conjugated in the animal body to produce inactive substances. (4) The activity of the drug may be markedly decreased in the presence of serum or tissue fluids.

Thus while neomycin is more active than streptomycin in the test tube, animal experiments reveal that streptomycin still produces more favorable results in experimental tuberculosis of guinea pigs. Aureomycin and chloromycetin, although producing a mild bacteriostatic effect in the test tube, have demonstrated very little activity in the experimental animal.

On the other hand, practically all of the agents which are active in the animal body do show at least a fair degree of antituberculosis effect in the test tube. The occasional exception may be a compound which may be altered *in vivo* into substances which are active *in vitro*.

A most important factor in the evaluation of any drug is its therapeutic index, which is the maximal tolerated dose divided by the minimal curative dose. A drug which may be given in very high dosage without producing toxic effects may thus be more effective *in vivo* than another drug much more potent in the test tube but which can be given only in small dosage due to its greater toxicity. It was necessary to give viomycin in amounts exceeding three times that used for streptomycin to produce similar results. However, if viomycin can be readily tolerated in such dosage, it may be considered to be just as effective an antibiotic *in vivo* as streptomycin.

STREPTOMYCIN

It has been amply demonstrated that streptomycin, when administered to guinea pigs intramuscularly, has marked antituberculous activity upon early, well established tuberculosis. Even when treatment is delayed until seven weeks after subcutaneous infection with virulent tubercle bacilli, adequate doses of streptomycin will eradicate all gross evidence of disease except local abscesses at the site of inoculation and perhaps in the contiguous lymph nodes. Results obtained with streptomycin in experimental tuberculosis of guinea pigs and mice are so constant that they may be used as a standard with which to compare new agents. Certain other aspects of streptomycin therapy in guinea pig tuberculosis will now be discussed.

It is generally agreed that a dosage of between 6,000 and 10,000 mc. gm. per day is sufficient to retard the progression of the infection, and that this dose is as effective when given once a day as when given twice a day, or every four hours around the clock. Table II represents composite figures of the average index of tuberculosis* in guinea pigs killed at the end of thirty-five and sixty-four days of treatment with varying doses of streptomycin, administered on different time schedules. Treatment was withheld until six weeks after infection.

From the results presented (Table II) one can see that there was no significant differ-

TABLE II
AVERAGE AMOUNT OF GROSS TUBERCULOUS DISEASE IN GUINEA PIGS SACRIFICED AFTER THIRTY-FIVE AND SIXTY-FOUR DAYS OF SM TREATMENT

Group	SM Dosage Schedule	Average Index of Tuberculosis† at 35 Days		Average Index of Tuberculosis at 64 Days	
1	30 mg./day in 6 and 4 injections*	6.2		3.2	
2	30 mg./day in 2 injections	5.0	5.2	3.2	3.1
3	30 mg./day in 1 injection	4.3		2.8	
4	15 mg./day in 6 and 4 injections*	6.7		2.7	
5	15 mg./day in 2 injections	8.0	6.7	2.3	2.6
6	15 mg./day in 1 injection	5.2		2.8	
7	7.5 mg./day in 6 and 4 injections*	6.8		2.8	
8	7.5 mg./day in 2 injections	8.8	7.3	4.2	3.2
9	7.5 mg./day in 1 injection	6.3		2.7	
10	1 mg./day in 1 injection	11.8		12.5	

* Total daily amount divided into six equally spaced doses for the first thirty days, four doses per day thereafter.

† Each organ (lungs, liver, spleen, lymph nodes) was assigned a value of from 0 to 4+ according to the amount of gross tuberculous disease. Maximum total for each animal was therefore 16. Index of tuberculosis is the average total for each group.

* This index is used to compare the amount of gross tuberculous disease in different animals or groups of animals. For explanation see footnote of Table II.

ence, for each daily dosage, between the animals which had received it in multiple injections and those which had received only a single daily dose. At the thirty-five-day period of treatment it appears that the animals receiving 30 mg. a day had slightly less disease than those treated with 15 mg. a day, and the latter group had slightly less disease than the groups treated with 7.5 mg. a day. At the sixty-five-day period, however, even this slight difference is not apparent, as the groups treated with 7.5 mg. a day presented about the same picture as those treated with 30 mg. a day. It is obvious that 1 mg. of streptomycin daily was not sufficient to hold the disease in check.

It has been noted[17] that even when streptomycin is administered to guinea pigs, starting prior to infection, it will not prevent the dissemination of tubercle bacilli from the local site of inoculation, and the infection will progress until necrosis and hypersensitivity appear. From then on the course of the infection becomes regressive. Since it is known that hypersensitivity and acquired resistance appear at about the same time in the guinea pig, it is assumed that the animals at this time will have developed some resistance, which seems to be a necessary factor, in conjunction with streptomycin, to prevent the multiplication of the tubercle bacilli and halt the progression of the disease.

Early in our studies we noted that although the lungs, liver, spleen and lymph nodes in guinea pigs were free of gross tuberculosis there often remained at the site of inoculation large abscesses containing many acid-fast organisms (at times the inguinal and iliac nodes draining the site also had abscesses), even though treatment with therapeutic doses of streptomycin was given without interruption for as long as 528 days. It was likewise noted that when these abscesses discharged spontaneously or were drained, healing was then likely to occur, leaving only a residual fibrous scar. This observation suggested that streptomycin had little effect on a non-sloughing abscess and that experiments should be designed to study the effect of streptomycin upon chronic tuberculous lesions in guinea pigs. At the same time it could be determined whether there would be a difference in the streptomycin sensitivity of the organisms isolated from such lesions as contrasted to the sensitivity of the organisms recovered from early, acute disease.

The following two methods of inducing chronic tuberculosis in guinea pigs may be used: (1) enhancement of the low native resistance of the animal by vaccination with living attenuated tubercle bacilli, such as BCG (Bacillus Calmette Guérin) or H37Ra, a rough avirulent strain of human tubercle bacilli, prior to infection with virulent organisms; (2) by treating an already established disease with therapeutic doses of streptomycin for a limited time; when treatment with this drug is discontinued slow, progressive tuberculosis develops in the animals which is characterized by abscesses and cavity formation.

We are aware that the type of necrotic lesion produced in guinea pigs is not identical with that observed in humans. The lesions produced in guinea pigs are characterized by small abscess formation, fluid in nature, and composed of many intact polymorphonuclear leukocytes. In humans, however, the necrotic areas are caseous and cheese-like, and in contrast to the lesions in guinea pigs the cells have disintegrated and are not likely to slough as readily.[18]

Therefore, it was postulated that if streptomycin had no effect upon such lesions in guinea pigs, one should not anticipate favorable results in humans with chronic forms of tuberculosis in whom many of the necrotic lesions are firm and non-sloughing.

Accordingly, the following experiment was undertaken: Chronic cavitary and nodular disease was produced in a group of guinea pigs weighing between 500 and 700 gm. by infecting them subcutaneously with the H37Rv tubercle bacilli one year after vaccination with H37Ra microorganisms. Animals sacrificed from time to

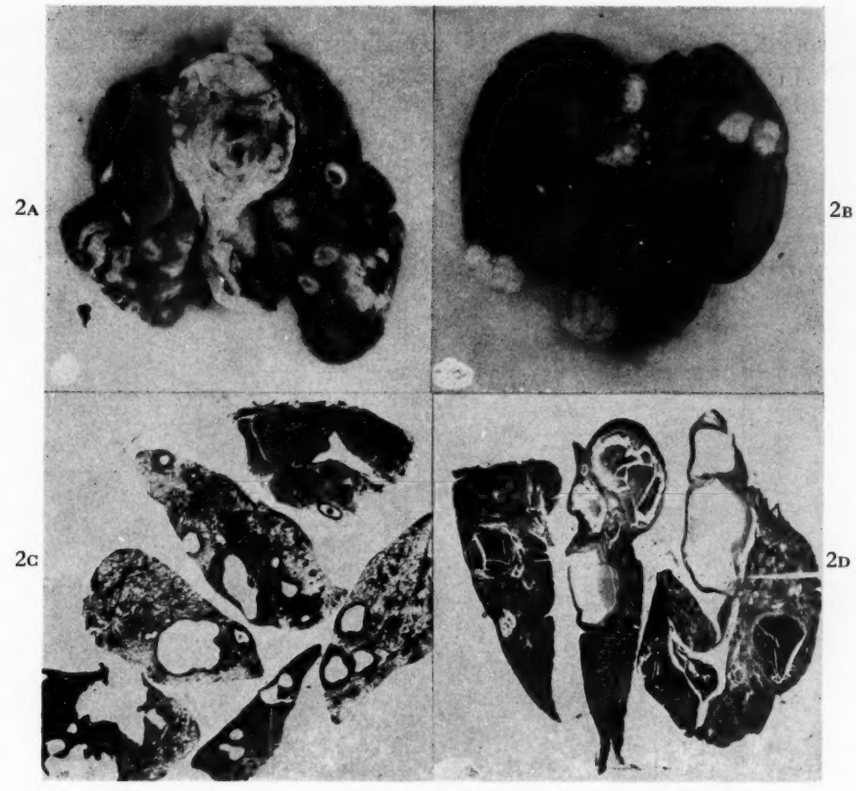

FIG. 2. Photographs of lung and liver from guinea pig with chronic tuberculosis treated 300 days with streptomycin. A, lung showing thin-walled cysts; B, liver showing thicker walled cystic abscesses; C, cut sections from same lung; D, cut sections through same liver.

time confirmed the development of slowly progressive, nodular tuberculous lesions. When this chronic disease had been allowed to progress for ninety-six days, treatment with therapeutic dosage of streptomycin was begun and continued without interruption for 300 days. Of the seven animals that were sacrificed at this time three demonstrated gross lesions. (Fig. 2.) The liver, spleen and lungs contained cyst-like abscesses filled with serous fluid containing myriads of acid-fast bacilli. In the lungs these cysts were thin-walled, but the lesions in the spleen and liver usually had a thicker fibrous wall. Virulent tubercle bacilli could be cultured abundantly from these lesions, and cultures obtained from five different organs exhibited no significant increase in resistance to streptomycin.

The other four guinea pigs sacrificed at this time showed very little tuberculous disease grossly. It could be said that streptomycin treatment in these animals had favorably influenced the chronic, nodular lesions presumably present at the start of treatment. However, it is important to realize that although a sample of six guinea pigs was sacrificed on the ninety-sixth day after infection and they all showed the presence of generalized, nodular-type disease, this does not necessarily mean that these four guinea pigs also had such lesions. Some degree of variation in the type and extent of disease may be expected in any large group of guinea pigs. At any rate, the uniformly excellent effect that streptomycin produces on the acute type of disease in guinea pigs was lacking, and the chronic lesions persisted and even progressed in three of seven animals despite the administration of streptomycin and the persistence of drug-sensitivity of the tubercle bacilli.

Streptomycin Resistance. It is a well known fact that tubercle bacilli may easily be

TABLE III

SENSITIVITY TO STREPTOMYCIN OF TUBERCLE BACILLI ISOLATED FROM TREATED GUINEA PIGS

Guinea Pig	Group*	Days of SM Therapy	Days Off SM before Death	Culture of†	Results of Streptomycin Sensitive Tests					
					Direct Isolation on Solid ATS Medium mc. gm./ml.				Subculture Tested in Liquid Medium mc. gm./ml.	
					0	3.5	15	200	Resistant	Sensitive
15 pigs	1–10	35	0		1 colony to 2+	0	0	0		1.0
761	9	35	0		2+	0	0	0	2.5	5.0
7 pigs	3–10	60–64	0		1 colony to 2+	0	0	0		1.0
152	2	90	65	Spleen cyst	2+	0	0	0		
				Organs	30	0	0	0	1.0	2.5
99	9	90	63		4 colonies	0	0	0		0.5
531	8	90	87	Liver	6 colonies	0	0	0		0.5
				Lung and spleen	2+	0	0	0		0.5
850	8	90	89		35	0	0	0		0.5
859	9	90	89		25	0	0	0		0.5
58	8	90	121		1+	0	0	0		0.5
355	5	90	157		3+	0	0	0	0.5	1.0
843	7	90	181		9 colonies	0	0	0		0.5
419	5	90	219	Lung	35	0	0	0		0.5
				Liver and spleen	10	0	0	0		0.5
466	2	90	225		50	0	0	0		0.5
844	7	90	243		2 colonies	0	0	0		0.5
528	3	90	271		45	0	0	0		0.5
776	3	90	281		45	0	0	0		0.5
367	2	90	303		1+	0	0	0		0.5
353	3	90	310		2+	0	0	0		0.5
680	5	90	336		1+	0	0	0		0.5
599	10	92	0		16	0	0	0		1.0
845	7	135	0	Liver abscess	3+	0	0	0		
851	8	136	0		2 colonies	0	0	0		0.5
618	1	180	35		23	1 colony	0	0	1,000	
18	7	180	83		2+	0	0	0	0.5	1.0
628	1	180	89		45	0	0	0	1.0	2.5
343	7	180	93		4+	2+	1+	0	10	30
378	1	180	106		1+	0	0	0	1.0	2.5
869	1	180	106		2+	0	0	0		0.5
517	4	180	139		12	0	0	0		0.5
160	7	180	180		40	0	0	0		0.5
648	7	180	235	Lung	40	0	0	0		0.5
863	10	201	0	Organs 1	5 colonies	0	0	0		0.5
				Organs 2	3 colonies	0	0	0		0.5
832	8	219	0		7 colonies	0	0	0	1.0	2.5
423	8	225	0		9 colonies	0	0	0	1–2.5	2.5–5
384	10	262	0		35	0	0	0		0.5
726	1	283	0	Spleen	19	0	0	0	1–2.5	2.5–5
				Liver	6 colonies	0	0	0	2.5	5.0
				Lung	10	0	0	0	1.0	2.5
860	9	294	0		2+	0	0	0		0.5
157	6	298	0		1+	0	0	0	2.5	5.0

TABLE III (*Continued*)

Guinea Pig	Group*	Days of SM Therapy	Days Off SM before Death	Culture of†	Results of Streptomycin Sensitivity Tests					
					Direct Isolation on Solid ATS Medium mc. gm./ml.				Subculture Tested in Liquid Medium mc. gm./ml.	
					0	3.5	15	200	Resistant	Sensitive
87	6	313	0	Liver abscess	3+	0	0	0	1.0	2.5
				Organs	2+	0	0	0	1.0	2.5
403	1	314	0		20	17	6 colonies	0	60	250
846	8	316	0		5 colonies	0	0	0	1.0	2.5
370	6	325	0		20	0	0	0	1.0	2.5
396	7	348	0		5 colonies	0	0	0	1.0	2.5
759	6	354	0	Spleen	1+	0	0	0	1.0	2.5
243	3	367	0		1+	0	0	0	1.0	2.5
842	7	390	0		1+	0	0	0	0.5	1.0
755	9	394	0		10	0	0	0	1.0	2.5
388	3	399	0		10	0	0	0	1.0	2.5
687	4	401	0	Subinoculum‡	2+	0	0	0	1.0	2.5
516	2	412	0		2+	1 colony	0	0	2.5	5.0
571	8	487	0		8 colonies	0	0	0		0.5
612	4	562	0		1 colony	0	0	0	0.5	1.0
281	2	567	0		1 colony	0	0	0	0.5	1.0
32	6	573	0		20	0	0	0	1.0	2.5
770	5	573	0		1+	0	0	0	0.5	1.0
36	5	573	0		2+	0	0	0	2.5	5
94	4	573	0	Subinoculum	2+	0	0	0	1.0	2.5
853	9	573	0	Subinoculum	1+	0	0	0		0.5

* Groups 1, 2 and 3 received 30 mg. streptomycin daily
4, 5 and 6 received 15 mg. streptomycin daily
7, 8 and 9 received 7.5 mg. streptomycin daily
10 received 1.0 mg. streptomycin daily

† Unless otherwise indicated, disease-containing portions of the lungs, liver, spleen and lymph nodes (these four tissues also referred to as "organs") were ground up together for culture.

‡ Culture made from guinea pig subinoculated with ground-up tissues.

made resistant to streptomycin by *in vitro* cultivation in artificial media containing the drug. Drug-resistant strains of tubercle bacilli often emerge in humans under treatment with streptomycin, and it has been shown by Youmans[19] and Hobby[20] that streptomycin-resistant organisms may be cultured from mice treated with the drug. The ease with which microorganisms become resistant to the action of streptomycin constitutes one of its main drawbacks.

At this time we should like to present some preliminary data on the streptomycin sensitivity of tubercle bacilli isolated from guinea pigs treated with streptomycin for varying periods of time and under different time schedules. Tubercle bacilli were recovered from seventy-eight guinea pigs that were grouped and treated as shown. (Table III.) Various types of lesions from different organs were removed at autopsy from each animal, ground with sterile sand, digested with 3 per cent sodium hydroxide, homogenized, concentrated, and planted on solid ATS medium containing varying concentrations of streptomycin. Growth from the control tube was also subcultured in Tween-albumin liquid medium and this

subculture tested for streptomycin sensitivity in liquid medium. The results are presented in Table III.

The most striking finding from these tests is the infrequency with which streptomycin-resistant cultures, and even isolated resistant variants, were recovered. Only three of the seventy-eight animals (618, 343 and 403) yielded definitely resistant cultures. Fourteen animals were treated continually for over a year, and none of these yielded a resistant culture. With but few exceptions, those animals treated for less than 200 days gave cultures which were still sensitive to 0.5 or 1.0 mc. gm. per ml. in liquid medium or, in other words, were just as sensitive as the original H37Rv culture used for infection. The majority of the cultures isolated from guinea pigs treated for over 200 days, however, proved to be sensitive to 2.5 to 5.0 mc. gm. per ml., representing a two- to fivefold increase in resistance.

Response to Streptomycin Therapy of Animals Infected with Streptomycin-resistant Microorganisms. To answer the question of whether *in vitro* resistance corresponded to *in vivo* resistance, investigation was made of the effect of streptomycin in experimental tuberculosis produced by drug-resistant strains. It was uniformly observed that in guinea pigs and in mice infections produced by highly resistant microorganisms, whether from streptomycin-treated patients or from laboratory strains, did not respond to treatment with the drug.[21–23]

When guinea pigs were infected with strains exhibiting varying degrees of streptomycin resistance[23] and therapeutic doses of the drug were administered, the results indicated that in general those cultures which proved to be resistant to 15 or more mc. gm. per ml., as tested in Tween-albumin liquid medium, produced non-responsive infections whereas those sensitive to 2.5 or less. mc. gm. per ml. usually produced infections amenable to the drug. There were, however, some inconsistencies in the response of the guinea pigs infected with a few cultures of borderline resistance.

Another interesting observation in this study was the finding that the strains of tubercle bacilli used to infect these animals showed no appreciable increase in drug resistance when recovered at the end of the experiment from the streptomycin-treated animals. One might have expected that strains which already possessed some degree of resistance would show an increase in resistance while residing and multiplying in the streptomycin-treated pigs.

PARA-AMINOSALICYLIC ACID

In contrast to the excellent and reproducible results obtained with streptomycin in experimental tuberculosis of mice and guinea pigs the published results of various investigators with PAS reveal a strange inconsistency, not only among the different laboratories involved but also in repeated experiments of the same investigators. Youmans[24] in 1947 showed that treatment with PAS gave good results in white mice infected with the H37Rv culture. The drug was incorporated in the diet in a concentration of 2 per cent by weight. These good results were later confirmed by Moeschlin[25] while Levaditi[26] and Duca[27] reported only fairly good results in the treatment of murine tuberculosis by PAS. Swedberg,[28,29] on the other hand, has reported both successful and unsuccessful experiments with white mice. In one experiment in particular when ten strains of human tubercle bacilli varying in *in vitro* sensitivity to PAS were used to infect white mice, no beneficial effect from the treatment of PAS was seen in the mice infected with five definitely resistant strains, whereas with mice infected with four sensitive strains a very good increase in mean survival time was obtained. In the other strain, however, which *in vitro* was actually the most sensitive of all, no increase in survival time was obtained. Swedberg[29] also reported that by administering 50 mg. of PAS per day in the drinking water a blood concentration up to 9 mg. per cent was obtained in the mice. Even with these large doses, however, and continual treatment for 110 days, all the treated mice eventually died with massive tuberculosis,

the best mean survival time being sixty-six days.

The situation in respect to experimental guinea pig tuberculosis is even more confusing. Feldman, Karlson and associates of the Mayo Clinic have reported four different experiments with PAS.[30–32] In the first experiment no beneficial effect was noted, and the authors blame this poor result on the fact that the period of treatment was only forty-eight days. In their second experiment in which treatment with 4 per cent PAS in the diet was started forty-two days after infection and continued for 119 days, excellent results were obtained despite the fact that determination of the blood levels of PAS showed them to be less than 0.5 mg. per cent. Another experiment in which PAS was again given in a concentration of 4 per cent in the diet for 133 days starting twenty-five days after infection, showed fairly good results. The treated animals demonstrated only about one-third of the amount of tuberculous disease as the controls. In the fourth trial PAS was given by subcutaneous injection once a day for forty-nine days, and excellent results were obtained. The beneficial effect was just as good in those animals treated with 125 mg. per day as in those treated with 500 mg. per day. Unfortunately, no determination of the blood levels of PAS was given for the guinea pigs in this experiment or the preceding one.

Bloch[33] administered the sodium salt of PAS subcutaneously and after only twenty-four days of treatment found that those animals treated with PAS had less disease than those treated with streptomycin. McClosky and Smith,[34] on the other hand, administered PAS orally in the maximum tolerated dosage of 500 mg. per kg. per day for eleven weeks and despite the fact that the blood levels obtained under this regimen were good (10 to 20 mg. per cent for five hours after a single dose of 500 mg. per kg.), very little beneficial effect from the PAS treatment was noted. The results were poorer than those obtained with the sulfones and no indication of potentiation was obtained with the combination of PAS and streptomycin, while a definite potentiating effect was demonstrated with the combination of a sulfone drug and streptomycin. Moeschlin[35] also reported that in experimental tuberculosis of guinea pigs he found that PAS was less effective than promin. Bogen[36] states that when PAS is given orally to guinea pigs, 100 mg. per day is ineffective, 500 mg. per day is only partially effective, and it is necessary to give as much as 2 gm. per kg. per day before good results are obtained.

Both McClosky using rabbits,[34] and Swedberg using white mice[28] reported no results from PAS treatment when the infecting organism was the bovine strain, Ravanel. This is not entirely unexpected since it is known that at least some strains which are labeled Ravanel are highly resistant to PAS *in vitro*.

In the Trudeau Laboratory results obtained in experimental guinea pig tuberculosis with PAS have been relatively poor. In our first experiment the neutralized liquid form of sodium PAS was given by mouth in dosage of 400 mg. per kg. per day for 110 days. At the end of this time the treated animals demonstrated about 70 per cent of the amount of disease seen in the control animals. In another experiment the guinea pigs were given powdered PAS in capsules by mouth in dosage of 500 mg. per kg. per day for fifty days. The treated animals in this experiment showed 78 per cent of the amount of disease that was seen in the control animals. Determinations of PAS in the blood revealed that the level varied from a low of 0.2 to a high of 1.5 mg. per cent. In both these experiments the infecting organism was the virulent human strain H37Rv, which is highly sensitive to the action of PAS in the test tube. Tubercle bacilli isolated from eight guinea pigs treated for 110 days with PAS failed to reveal any increase in resistance to the drug *in vitro*. An attempt was made to give the drug subcutaneously or intramuscularly by neutralizing it with an appropriate amount of sodium bicarbonate and dissolving it in water, but this product could not be toler-

ated as it produced large areas of necrosis after only a few days of administration.

Bacterial Resistance to PAS. Strains of tubercle bacilli resistant to PAS may be produced *in vitro*, although this is much more difficult than the induction of resistance to streptomycin. After repeated transfers in Tween-albumin medium containing PAS for a period of approximately eight months we were able to produce a strain of H37Rv which was resistant to at least 50 mg. per cent of PAS. Resistance of this culture to PAS has been retained after thirty transfers in Tween-albumin medium without PAS. Karlson[37] and Swedberg[29] have already reported that animals infected with strains showing *in vitro* PAS resistance do not respond to adequate treatment with PAS. We also have found that guinea pigs infected with this H37Rv strain made resistant to PAS, as well as with several PAS-resistant cultures isolated from patients treated with the drug, do not demonstrate any beneficial effect from the administration of the drug.

Contrary to the early reports in the literature, strains of tubercle bacilli showing *in vitro* resistance to PAS may frequently be obtained from patients under treatment with this drug. Karlson[38] has presented a review of the literature on this subject. We have demonstrated such a PAS-resistant strain from the sputum of a patient who had been taking the drug for only sixty days before the isolation was made.

STREPTOMYCIN PARA-AMINOSALICYLATE

Streptomycin or dihydrostreptomycin* can be combined with PAS to form a salt which is soluble in water. The preparation used in these experiments was dihydrostreptomycin-para-aminosalicylate containing approximately 725 mg. of PAS for each gm. of pure dihydrostreptomycin base. Table 1 shows that an amount of this compound equivalent to 0.25 mc. gm. of pure dihydrostreptomycin per ml. of medium was sufficient to inhibit growth of tubercle bacilli. Against the streptomycin-resistant strain of H37Rv the salt was active in concentration of 1.0 mc. gm. per ml. but here, as with PAS alone in liquid medium, the end point of inhibition is not sharp, the tubes containing 1, 10 and even 100 mc. gm. per ml. showing a very slight degree of turbidity. It will be noted that the tube containing 1 mc. gm. per ml. also has 0.7 mc. gm. per ml. of PAS, which is in itself sufficient to inhibit growth of the H37Rv strain.

Hobby[39] reported that streptomycin-para-aminosalicylate was more effective in tuberculosis of mice than the same quantity of streptomycin sulfate. A comparison between streptomycin alone and dihydrostreptomycin-para-aminosalicylate in the treatment of experimental tuberculosis in guinea pigs has been made in the Trudeau Laboratory. The animals were infected subcutaneously with 0.002 mg., dry weight, of the H37Rv culture, and treatment was started twelve days after infection. Twenty-five animals were treated with streptomycin alone, fifteen with 5,000 mc. gm. per day and ten with 10,000 mc. gm. per day. A similar group of animals was treated with dihydrostreptomycin-para-aminosalicylate. Twelve animals remained untreated. After ninety-two days of treatment a representative number of animals from each group was sacrificed and revealed the following average indexes of tuberculosis: streptomycin, 5,000 mc. gm. per day, 1.8; 10,000 mc. gm. per day, 1.6; dihydrostreptomycin-para-aminosalicylate, 5,000 mc. gm. per day, 1.5; 10,000 mc. gm. per day, 1.5. The control untreated animals had 13.4.

The remaining animals were kept on treatment for a total of 195 days; then therapy was discontinued to note the relapse rate. After another 125 days all surviving animals were sacrificed. During this period without treatment several animals from each group died either accidentally or of intercurrent infection, but none of them died of tuberculosis. The untreated control

* Dihydrostreptomycin is similar to streptomycin in its tuberculostatic and tuberculocidal effects both *in vitro* and *in vivo*. Streptomycin-resistant strains are also resistant to dihydrostreptomycin.

animals had all died of tuberculosis by this time. At the termination of the experiment the various groups revealed the following average indexes of tuberculosis: streptomycin, 5,000 mc. gm. per day, 6.3; 10,000 mc. gm. per day, 3.8; dihydrostreptomycin-para-aminosalicylate, 5,000 mc. gm. per day, 3.5; 10,000 mc. gm. per day, 3.5.

It may thus be seen that after ninety-two days of treatment the results obtained with streptomycin alone were approximately the same as those with dihydrostreptomycin-para-aminosalicylate. When the animals were killed 125 days following a 195-day course of treatment, there was also no essential difference between the group of animals treated with 10,000 mc. gm. of streptomycin and 10,000 mc. gm. of dihydrostreptomycin-para-aminosalicylate. In the groups treated with only 5,000 mc. gm. a day (a dose which is under the borderline of maximum effect for streptomycin alone), however, the animals treated with streptomycin alone showed a little less than twice the amount of tuberculosis as those treated with the PAS salt of dihydrostreptomycin.

This slight superiority of the PAS salt over streptomycin alone is much less than that seen by other observers when full doses of PAS were given by mouth along with sub-effective doses of streptomycin.[31,33,35,40] Blood levels of PAS in guinea pigs given 10,000 to 20,000 mc. gm. of dihydrostreptomycin-para-aminosalicylate salt were insignificant. The peak level was 0.5 mg. per cent or less, and by the end of two and a half hours no PAS was demonstrable. Recent data suggest that the amount of PAS in the salt is not enough to delay the emergence of streptomycin-resistant tubercle bacilli in patients taking this salt, a delay which does occur when full doses of PAS are taken by mouth along with streptomycin taken intramuscularly.[41]

NEOMYCIN

The inhibition of growth of the tubercle bacillus by neomycin was first reported by Waksman and his co-workers,[42] and these studies have been extended by Hobby,[43] Rake,[44] Felsenfeld,[45] and Steenken and Wolinsky.[46] Table 1 shows that neomycin is slightly more effective than streptomycin in inhibiting the growth of the H37Rv strain and that it is equally active against the streptomycin-resistant microorganisms. The presence of serum in the medium interferes slightly with this action. Neomycin has been found to be effective in experimental tuberculosis of both mice and guinea pigs.

Guinea pigs treated for forty-eight days with 10,000 units per kg. per day, intramuscularly, revealed an average index of tuberculosis of 4.6 as compared to the untreated controls with 10. Animals infected with the streptomycin-resistant strain of H37Rv, and similarly treated with neomycin, revealed an index of 3.3 as compared to the untreated controls with 11. Another group of guinea pigs treated with neomycin, 10,000 units per kg. per day for forty-eight days, followed by 20,000 units per kg. per day for forty-nine days, revealed an average index of tuberculosis of 6.1 as compared to the untreated controls with an index of 13.6. In animals infected with the streptomycin-resistant strain and treated similarly, the index was found to be 2.7 as compared to the controls with 12.2. A comparison series of animals infected with the H37Rv streptomycin-sensitive culture and treated with streptomycin, 20,000 mc. gm. per kg. per day, revealed slightly but definitely less tuberculous disease in all cases than the neomycin-treated guinea pigs. In the doses used neomycin exhibited a slight toxicity for the guinea pigs, producing progressive weight loss.

A strain of H37Rv microorganisms resistant to 1,000 units of neomycin per ml. was produced after seven transfers in Tween-albumin liquid medium containing increasing concentrations of the drug. Little increase in resistance was manifested until the fourth transfer, but each succeeding passage produced a rapid rise in resistance. A similar series of transfers of the same organism at the same time in streptomycin-containing liquid medium produced

a culture resistant to 1,000 mc. gm. of streptomycin per ml. after six passages.

Neomycin, therefore, may be considered to be a potent antituberculous agent both in the test tube and in the experimental animal. Although slightly more active *in vitro*, it is nevertheless definitely less active than streptomycin in the guinea pig. The neomycin produced at this time, however, is a mixture of at least three separate antibiotic substances.[47] It may be hoped that with further purification more antituberculous activity and less toxicity will be the result.

THIOSEMICARBAZONES

This group of compounds was introduced by Domagk and co-workers[48] in 1946. In liquid media a concentration of between 0.5 and 1 mc. gm. per ml. of TB-1 (4-acetylaminobenzaldehyde thiosemicarbazone) is sufficient to inhibit growth of tubercle bacilli. Good results in the treatment of murine tuberculosis with TB-1 have been reported by Levaditi[49] and Donovick.[50] Karlson and co-workers[51] have reported the effect of this drug in experimental tuberculosis of guinea pigs. The drug was incorporated in the diet so that the animals received about 50 mg. of the drug per kg. per day for thirty-three days, followed by 100 mg. per kg. per day for twenty-seven more days. This dosage was well tolerated. The treated animals exhibited an average index of infection of 25.3, as compared with the untreated controls with 78.3, and another group of animals treated with therapeutic dosage of streptomycin, with 9.2. In our own experiments TB-1 (Tibione) was given to guinea pigs by mouth once daily in doses of 80 mg. per kg. per day for forty-eight days, starting twenty-six days after infection with the H37Rv culture. A definite retardation of the tuberculous process was noted, this beneficial effect being about one-half of that accomplished with therapeutic dosage of streptomycin and definitely greater than that achieved with PAS in doses of 500 mg. per kg. per day.

The application of TB-1 in clinical tuberculosis is limited by its marked toxicity. A dose corresponding to the amount used in experimental animals would be about 3 gm. per day to a human being whereas doses in excess of 200 mg. per day are excessively toxic. Extensive investigations of other members of the thiosemicarbazone group and related compounds are being carried out by Hoggarth et al.[52] and Donovick et al.[53,54] It is to be hoped that a less toxic and more efficient antituberculosis agent may be forthcoming from this group of compounds.

SULFONES

Under the heading of sulfones are included diamino-diphenylsulfone, promin, diasone, promizole, sulfetrone and other derivatives of diamino-diphenylsulfone. A sulfone drug was actually the first chemotherapeutic agent to produce an unquestionable retarding effect in experimental tuberculosis. This was demonstrated in 1940 by Rist and his associates.[55] Since that time extensive work has been carried out in many laboratories in an attempt to produce related compounds which would have greater therapeutic and less toxic effect. The reader is referred to the works of Youmans et al.,[56] Freedlander and French,[57] Hoggarth and Martin,[58] and Smith, Jackson and Bauer,[59] as well as the excellent general review by Feldman.[1] It is generally agreed that certain sulfone drugs when given in maximum tolerated doses produce a beneficial effect in experimental tuberculosis which is about one-quarter that of streptomycin. The main reason for the limited *in vivo* effect of the sulfones has been summarized by Smith[59] as follows: "The tuberculostatic activity of the sulfones thus far studied is too low in relation to the tolerated concentration that can be maintained in the blood and tissues of experimental animals, to produce complete eradication of tuberculous infections."

The significance of this may be better understood when it is realized that more promin cannot be given than the amount

required to produce a blood level of 5 to 10 mg. per cent in the experimental animal whereas 20 mg. per cent is necessary to inhibit growth in the test tube. Synergistic effect from the combination of streptomycin with several sulfones has been reported, however, in experimental tuberculosis, and a more suitable and less toxic compound may yet be produced to use along with streptomycin or other powerful antibiotic agent for the treatment of clinical tuberculosis.

SUBTILIN

This antibiotic, obtained from a certain strain of Bacillus subtilis, was introduced by Jansen and Hirschmann.[60] Subtilin inhibits the growth of tubercle bacilli in liquid media only if the organisms are growing in a dispersed fashion as they do in media containing a wetting agent such as Tween 80. In other liquid media, however, in which the growth occurs in aggregates, this antibiotic has very little if any inhibitory effect.[61,62]

Salle[63] was readily able to induce resistance to subtilin by serial transfer in drug-containing liquid media, of Mycobacterium phlei, Staphylococcus aureus and Esch. coli.

The *in vivo* antituberculous activity of subtilin has been the subject of conflicting reports in the literature.[62] We were unable to show any effect from the maximal tolerated dose of subtilin in tuberculous guinea pigs. In a recent communication Salle[64] emphasizes the fact that different samples of subtilin demonstrate great variation in their action on the tubercle bacillus. Using a batch of this drug with good antituberculous properties (and prepared in such a way that it became soluble in serum) in doses of 22 mg. per day, he showed a good effect in experimental guinea pig tuberculosis. The final word on subtilin must be postponed until a more purified and standard preparation is available.

STREPTOTHRICIN AND STREPTOTHRICIN VI

Streptothricin is an antibiotic which was described by Waksman and Woodruff[65] in 1942. It was found to have fairly good tuberculostatic activity *in vitro*. (Table 1.) Because of its extreme toxicity, however, it proved to be unsatisfactory in the treatment of experimental tuberculosis in guinea pigs and hamsters. Even in doses sufficient to produce marked toxic effects, Feldman and Hinshaw[66] as well as Steenken and Wagley[67] found streptothricin to be ineffective.

Streptothricin VI was introduced by Waksman et al.[68] in 1949. This was the sixth antibiotic preparation of the streptothricin complex isolated from a strain of Streptomyces lavendulae. Table 1 shows that this antibiotic inhibits the growth of tubercle bacilli in a concentration of 5 units per ml. and that it is also active against streptomycin-resistant microorganisms. In the guinea pig, however, this antibiotic also shows too great a toxicity. In the maximum tolerated doses of 400 units per day it was ineffective in experimental tuberculosis of this animal. If less toxic preparations of this antibiotic can be produced, an adequate test of its *in vivo* effect may be made.

AUREOMYCIN

Table 1 reveals that aureomycin produces definite inhibitory effect on the growth of tubercle bacilli in concentration of 10 mc. gm. per ml. The presence of serum in the medium, however, interferes appreciably with this inhibitory action. Despite its bacteriostatic effect in the test tube aureomycin has been found to be entirely ineffective in the treatment of experimental tuberculosis in animals. Steenken and Wolinsky[69] treated guinea pigs intramuscularly with 3.2 mg. per kg. per day for forty-eight days; Perry[70] treated guinea pigs with 10 mg. per kg. per day subcutaneously for forty-two days; Rake[71] treated mice by mouth with 100 mg. per kg. per day. In none of these experiments was any beneficial effect demonstrated. Aureomycin has also been given to patients with pulmonary tuberculosis without producing any improvement.[72,73]

CHLOROMYCETIN

Chloromycetin has a weak tuberculostatic effect *in vitro* which requires from 20 to

40 mc. gm. per ml. for growth inhibition. Youmans and his associates[74] found that this antibiotic had only a very slight suppressive effect on tuberculosis of mice, and Carr and his associates[75] found it to be entirely ineffective in the treatment of guinea pig tuberculosis although given in the diet in concentration of 0.5 per cent. It is fairly definite, therefore, that chloromycetin is of no value as an antituberculosis agent.

PENICILLIN

The *in vitro* effect of penicillin on the tubercle bacillus has recently been studied and the literature reviewed by Kirby[76] and Solotorovsky.[77] Depending upon the size of the inoculum, penicillin inhibited the growth of mycobacterium tuberculosis in concentrations varying from 1 to more than 200 units per ml. of Tween-albumin liquid medium. In other types of media, however, in which the growth occurs in aggregates, penicillin even in very high concentrations had very little effect on the growth of tubercle bacilli. In the experimental animal penicillin had no effect on the course of tuberculous disease, as reported by Hauduroy[78] and Dickinson.[79] In this laboratory we have treated guinea pigs with the maximum tolerated dose of penicillin (which was found to be 1,000 units per kg. per day for forty-five days) with completely negative results. Guinea pigs are unable to tolerate doses any higher than this.

ORTHO-AMINOPHENOL

O-aminophenol has been described by Japanese workers[80] as having good antituberculosis properties *in vitro* and *in vivo*. Table 1 reveals that this drug, in a concentration of 10 mc. gm. per ml., has the power to inhibit the growth of tubercle bacilli in the test tube. A glance at Figure 1, however, reveals that it has only feeble activity in the guinea pig. Twenty-five guinea pigs weighing between 500 and 800 gm. were infected subcutaneously with 0.15 mg. of the H37Rv culture, and treatment was started nine days later. Ten animals were treated with o-aminophenol hydrochloride, intramuscularly, 50 mg. twice a day for twenty-one days, then 50 mg. once a day until fifty-five days after infection, when all survivors were killed. The index of tuberculosis in the controls was found to be 11.8, while that for the guinea pigs treated with o-aminophenol was found to be 8.7. The drug produced extensive tissue necrosis at the site of injection. Ten animals treated with streptomycin, 10,000 mc. gm. intramuscularly once a day, showed an index of tuberculosis of only 1.4. It is thus apparent that o-aminophenol offers very little promise as an antituberculosis agent.

TERRAMYCIN

Terramycin is a new antibiotic agent produced by the mold Streptomyces rimosus, introduced by Finlay and associates.[81] Table 1 shows that it has the ability to inhibit the growth of tubercle bacilli in a concentration of 5 mc. gm. per ml., and that it is also active against the streptomycin-resistant microorganisms. Its action is inhibited to a mild degree by the presence of serum.

When this antibiotic was administered to guinea pigs, it was found to be quite toxic, in contrast to its lack of toxicity in humans. Guinea pigs were unable to tolerate more than 5,000 mc. gm. per kg. per day by intramuscular injection. Tuberculous animals infected subcutaneously and treated with terramycin intramuscularly in these relatively small doses for forty-eight days demonstrated less than half the amount of tuberculous disease of the control animals. Guinea pigs infected with the streptomycin-resistant strain of the H37Rv culture responded just as well to treatment with terramycin as those infected with the streptomycin-sensitive strain.

To confirm these results another group of guinea pigs was infected intraperitoneally with the H37Rv streptomycin-resistant organisms. Twenty animals were started on treatment with terramycin two days after infection, one half being given the drug in solution by mouth and the other half receiving the drug by intramuscular injection.

The dose was 5,000 mc. gm. per kg. per day for both routes of administration. Animals sacrificed twenty-six days after infection again revealed that terramycin treatment had definitely retarded the progress of the disease. The untreated animals exhibited a tuberculosis index of 8.5; animals treated with terramycin by mouth exhibited an index of 4.8; and those treated with terramycin intramuscularly had an index of 3.8. It is quite probable that if larger doses of the drug could be given to guinea pigs a greater beneficial effect might be demonstrated.

Terramycin also produced a definite beneficial effect in tuberculosis of mice. Intravenously infected C-57 black mice were treated with 250,000 mc. gm. per kg. per day, subcutaneously, for twenty-eight days, starting the day after infection. All survivors were killed after twenty-eight days. Among the control untreated mice the mortality was 80 per cent whereas the treated mice showed a mortality of 40 per cent. The control animals exhibited large tuberculous areas occupying most of the lung tissue and very many acid-fast bacilli could be seen in smears of the lung and spleen. On the basis of 0 to 4^+ the average amount of disease in the lungs of the control animals was considered to be 3.5. In the treated group the lungs of the mice that died early showed only a few scattered tiny gray areas, and the acid-fast bacilli in the smears of the lungs and spleen were not so numerous. The six treated mice killed on the last day exhibited an average involvement in the lungs of 0.9. All but one, however, showed many acid-fast bacilli in the lungs and spleen. These results in mice are similar to those reported earlier by Hobby.[82]

After five transfers in Tween-albumin medium containing terramycin no increase in resistance to the drug was exhibited by the H37Rv microorganisms.

VIOMYCIN

This new antibiotic is also produced by a member of the Streptomyces group and was first reported at the Ninth Streptomycin Conference of the Veterans Administration, April 1950.[82–84] It inhibits the growth of tubercle bacilli in concentration of 5 mc. gm. per ml., is just as active against streptomycin-resistant as it is against streptomycin-sensitive microorganisms, and its action is not interfered with by the presence of serum. In contrast to terramycin it proved to have very little toxicity for guinea pigs and could be given in doses as high as 150,000 mc. gm. per kg. per day.

A group of guinea pigs infected subcutaneously with the streptomycin-resistant H37Rv microorganisms was started on treatment with viomycin twenty-six days after infection. After forty-eight days of treatment all survivors were sacrificed. The control untreated group had an average index of tuberculosis of 11.4. The group treated with viomycin, 70,000 mc. gm. per kg. per day, had 3.5, while those treated with 35,000 mc. gm. per kg. per day, had 5.7. The results obtained with the higher dosage of viomycin were comparable to those seen with streptomycin, 20,000 mc. gm. per kg. per day in guinea pigs infected with streptomycin-sensitive tubercle bacilli.

Another group of pigs was treated with viomycin, 70,000 mc. gm. per kg. per day for forty-eight days, followed by 140,000 for thirty-eight days, after which all survivors were killed. These animals exhibited an index of tuberculosis of 1.9 in contrast to the untreated control group with an average of 13.3. A comparison group of animals treated with streptomycin, 20,000 mc. gm. per kg. per day for eighty-six days, showed an index of 2.0. It may, therefore, be concluded that treatment with viomycin produces beneficial effects in experimental tuberculosis of guinea pigs comparable to those seen with streptomycin, provided it is given in high enough dosage.

COMMENTS

It has been repeatedly emphasized by workers in this field that antibacterial therapy in tuberculosis differs from that in the common, acute infections because of the inherent nature of the pathologic process and the chronicity of the disease in man. It

soon became obvious that until an antibacterial agent is discovered which is powerful enough to eradicate all the tubercle bacilli in a short time, any such agent would have to be given for long period of time along with the established methods of treatment, such as bed rest, collapse therapy and surgery. During these prolonged periods of therapy the tubercle bacilli have an excellent chance to become resistant to the drug being given.

It is beyond the scope of this discussion to enter into the problem of the mechanism of development of such drug resistance. The reader is referred to reviews by Yegian[85] and Demerec.[86] It is interesting, however, to note that drug-resistant organisms are isolated from streptomycin-treated guinea pigs with acute or chronic tuberculous lesions only rarely, even after prolonged therapy, whereas mice and human beings seem to allow the production of such resistant strains readily. It is, therefore, evident that the guinea pig is not a suitable animal for studying methods for delaying the emergence of drug-resistant organisms. It would certainly be of great value in our understanding of this problem if this discrepancy could be explained.

For these reasons it is desirable that the preliminary acute tests of an antibacterial agent in guinea pigs and mice should be followed by long term experiments designed to note the effect of the agent on the chronic, caseous lesion. A few such experiments are described in the section on streptomycin. The action of various combinations of agents should also be tested with the double purpose of increased effectiveness and inhibition of the production of drug-resistant organisms.

Various types of streptomycin-dependent microorganisms have been described, including tubercle bacilli. For academic reasons this is a very interesting and important phenomenon, but it is of relatively minor significance clinically. Strains of tubercle bacilli whose growth is enhanced by the presence of streptomycin have been isolated very rarely from the sputum of patients treated with this drug. Theoretically, these strains should die out spontaneously as soon as streptomycin is discontinued. Dependence of tubercle bacilli on other antibiotic agents or other chemical agents has not as yet been described.

Streptomycin still remains the most potent antituberculosis agent at our disposal. Because of its two major drawbacks, neurotoxicity and development of drug-resistant strains, the search for other substances must continue unabated. PAS has demonstrated clinical usefulness, especially when given in combination with streptomycin, in increasing effectiveness and delaying the emergence of drug-resistant microorganisms. Neomycin and viomycin are both effective agents against experimental tuberculosis, but further information on the toxic potentialities for man must be had before a final evaluation of these drugs can be made.

SUMMARY

The study of antibiotic and chemical treatment in tuberculosis has been the subject of an increasing number of reports in the last few years. In this discussion we have attempted to summarize the experience gained in this laboratory in the testing of many antimicrobial agents for their antituberculosis effects. The methods for the preliminary *in vitro* and *in vivo* testing of new agents have been outlined, and the need for further evaluation of these agents in chronic, long term experiments has been brought out.

The relative antituberculous activities of the most promising substances have been outlined. Streptomycin remains the most powerful agent, but it possesses the two major drawbacks of neurotoxicity and drug-resistance. PAS, although producing variable results in experimental tuberculosis, has been found to be clinically useful, especially when given in combination with streptomycin. Neomycin and viomycin are new antibiotics which show good effect in the experimental animal and from which

further word is awaited in regard to toxicity for man.

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Antimicrobial Therapy in Human Tuberculosis*

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Tuberculosis is a disease which presents many unique problems to the physician, surgeon, pathologist, bacteriologist and in recent years to the pharmacologist as well. Most of these problems emerge from the fact that the course of tuberculosis is so remarkably prolonged in comparison with other infectious diseases, and this in turn is a product of the dogged perseverance of the causative bacillus and the conservative defensive attitude of the human host. Any therapeutic undertaking designed to cure tuberculosis will almost surely fail in a great majority of instances but treatment seeking merely to arrest the progress of an active infection is more likely to be successful, at least for the time being.

The physician who treats tuberculosis has great need for a simple, utterly dependable procedure which he may employ whenever activity of a tuberculous lesion is suspected. He needs an antimicrobial drug which will curb the invading bacillus continuously for many months, which may be repeatedly administered for prolonged courses, possibly over a period of years, and which is so dependable that its use will obviate the need for hospitalization, bed rest and surgery. This the physician does not have.

What progress toward the goal of an ideal antituberculosis drug can be recorded? Ten years ago it would have been said that no progress had been made because until 1940 there was no chemical method of arresting the multiplication of tubercle bacilli in the experimental animal or in man. It was in 1940 that some derivatives of diaminodiphenylsulfone were found which could bring established experimental tuberculous disease of guinea pigs to arrest. Five years later, in 1945, streptomycin was found capable of accomplishing similar arrest of such human disease as miliary tuberculosis. Scarcely more than one year ago did the combined use of streptomycin and para-aminosalicylic acid appear to become established as a means of producing more sustained antimicrobial therapy in clinical tuberculosis. However, the ideal of a completely bacteriostatic drug capable of fending off the progress of tuberculous disease indefinitely, without recourse to bed rest, collapse therapy and surgery, has by no means been attained in 1950.

Before therapy is undertaken the physician must be reasonably certain that active progressive tuberculous disease is present, for there is no object to the use of suppressive drugs if the disease is suppressed already. If we had access to a bactericidal drug, to a therapeutic agent capable of sterilizing tuberculous lesions *in vivo*, our approach would be utterly different. No such "magic bullet" is available or in prospect at this writing. Fortunately our diagnostic tools are sharper now than in previous years, for improved radiologic, bacteriologic, clinical and perhaps even serologic methods can be employed to ferret out active tuberculosis before it produces manifest illness.

Relations between drug therapy and other procedures

Rest therapy appears to remain a *sine qua non* in tuberculosis, especially when the

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disease involves such mobile organs as the lungs and the skeletal system. Present day antimicrobial drugs have not performed sufficiently well to justify any confidence that they may substitute for the proven "rest cure" and are therefore utilized in combination with bed rest, most frequently under hospital or sanatorium conditions.

In pulmonary tuberculosis collapse therapy measures are available which may greatly accelerate healing of many forms of active disease. Whether the choice be pneumothorax, pneumoperitoneum, phrenic nerve interruption, thoracoplasty or some other procedure may depend more upon the preference of physician and his geographic location than upon the nature and extent of the disease process. Many pages could be written regarding collapse therapy but each might well include the statement that drugs rarely substitute for collapse. Fortunately antimicrobial drug therapy and collapse therapy mutually supplement each other and, when judiciously employed together, each will enhance the accomplishments of the other.

The actual removal of tuberculous lung tissue was but rarely ventured prior to the time when a tuberculostatic drug could be utilized to prevent such postoperative complications as empyema and pulmonary dissemination of the disease. One of the most frequent contraindications to radical surgery which is encountered today is the presence of streptomycin-resistant tubercle bacilli resulting from what may have been injudicious or ill timed previous streptomycin therapy. A cardinal principle of antimicrobial therapy in tuberculosis is that its administration must be timed for strategic combination with other measures, especially with surgery. Too frequently drugs are administered in the hope of avoiding surgery; and when drugs fail, as they often do, surgery may be undertaken at increased risk or actually denied because drug-resistant bacilli have appeared.

CLINICAL EVALUATION OF NEW ANTITUBERCULOSIS AGENTS

The actual determination of the value of any proposed remedy against tuberculosis must be made on clinical grounds. Fortunately there are types of tuberculous disease which offer excellent and sometimes decisive evidence that a drug possesses therapeutic merit or even more definitely that it is devoid of clinical value.

Most types of pulmonary tuberculosis do not afford good material for judgment of a new drug because of the unpredictable course which many such lesions manifest even when untreated. When improvement occurs during treatment, it is by no means fair to assume that therapy produced the change. However, when new areas of disease appear by x-ray in formerly healthy appearing segments of lung despite therapy, more definite conclusions can be drawn. If even a few patients with pulmonary tuberculosis manifest progressive disease while under treatment with a new drug, that drug would appear unlikely to develop into a great remedy. When such progressive disease is delayed for several weeks or months, this might be due to the late appearance of drug-resistant strains of bacilli; but if the disease advances early in the course of treatment despite maximal safe doses of the drug, it is fair to conclude that the drug probably is impotent.

One should never attempt to judge the therapeutic value of a new drug which has been used only on patients with chronic or acute tuberculosis if this disease has proved refractory to previous treatment with effective drugs such as streptomycin and para-aminosalicylic acid. It is quite proper to treat such patients in the remote hope of making a contribution to their welfare and to secure needed pharmacologic information, but such studies do not constitute valid therapeutic trials of the substance in question.

Many physicians have noted repeatedly that the mucous membrane complications of pulmonary tuberculosis frequently are amenable to antibacterial therapy. Such diseases as tuberculous tracheobronchitis, laryngitis and enteritis provide excellent lesions for observation of therapeutic effects. Of these lesions, those which may be directly visualized are of greatest value,

especially ulcerating lesions of the larynx, mouth and tongue. Those which can be visualized through the bronchoscope are excellent for study if bronchoscopy is repeated frequently but tuberculous enteritis must be judged chiefly by clinical response. If these complications are not due to streptomycin-resistant bacilli, the subsequent use of streptomycin after failure of an experimental preparation will strongly emphasize the lack of value of the new drug under the conditions of its utilization. The results of effective antibacterial therapy in ulcerating tuberculous lesions of the respiratory tract mucosa appear so promptly that only a few weeks of treatment need be attempted. Furthermore, the favorable reaction of these lesions to such drugs as streptomycin is so consistent that no large number of cases would be required to determine if the new drug has a streptomycin-like effect.

The most clear-cut and crucial test of antituberculosis activity of a drug is its use in such extremely lethal forms of the disease as miliary tuberculosis and tuberculous meningitis. If any drug can establish a remission, even a temporary remission, in tuberculous meningitis or miliary tuberculosis, it immediately becomes a drug of tremendous clinical interest. Even if only a very few trials of the drug have yielded clear-cut results in such disease, its potentialities would appear to be very great and worthy of extensive study. However, if the new drug under trial should fail to influence the course of progressive disease of this character, the drug need not be excluded from further consideration. The accepted drug para-aminosalicylic acid, for example, usually does not of itself achieve arrest of tuberculous meningitis. At the present time no untried new drug could be used for treatment of tuberculous meningitis as the primary agent if streptomycin is available because such trial might jeopardize the patient's chance of recovery. At present streptomycin is available throughout most of the world in quantity sufficient to treat tuberculous meningitis and miliary tuberculosis. The usual conditions under which an experimental drug may be used freely in a highly fatal tuberculous disease would be either in combination with a streptomycin drug or in the treatment of an infection due to streptomycin-resistant bacilli. The latter circumstance presents itself all too frequently, especially in those countries where tuberculous meningitis is commonly encountered, and new drugs of promise should be dispatched to such countries for such study as soon as possible.

Tuberculous draining sinuses, especially those communicating with necrotic tuberculous lymph nodes and those associated with superficial cold abscesses, usually will close following treatment with effective antibacterial drugs and good drainage. Since this is a non-critical type of disease, only those drugs which surely lack dangerous toxic potentialities should be used; but if a consistent therapeutic response is observed quite promptly under such circumstances, the need for further clinical study of the drug is apparent.

One of the greatest uses for antimicrobial drugs in tuberculosis is for prophylaxis against the tuberculous complications of surgical procedures. This is especially true of pulmonary resection but is similar in the surgical treatment of genitourinary tract tuberculosis and in surgical treatment of tuberculosis of skeletal structures. Complications following such surgery are so infrequently encountered that the clinical evaluation of new drugs could not be achieved from observations made on surgical patients receiving prophylactic treatment unless complications were observed to occur, leading to serious doubt about the value of the drug in question.

PRACTICAL UTILIZATION OF AVAILABLE ANTITUBERCULOSIS DRUGS

The following drugs are now available, either as established antituberculosis drugs or as substances undergoing clinical investigation in tuberculosis: streptomycin, dihydrostreptomycin, para-aminosalicylic acid, Tbl-698 (also called tibione, myvizone,

conteben and so forth), promizole, promin, neomycin, terramycin and viomycin. Such an array of antimicrobial drugs is rather confusing, especially at a time when final evaluations have not been achieved for most of the substances named, but the author is willing to express his opinions regarding the present status of each and the trend of current investigation. It must be emphasized that opinions must always be tentative and subject to revision as new data are constantly being assembled in this most active field of therapeutic research.

Streptomycin and Dihydrostreptomycin. These two substances are the most thoroughly proven clinical remedies for use in tuberculosis, the most widely available, the most effective, and in many respects the most safe and convenient drugs to utilize in the treatment of many types of tuberculosis.

Streptomycin is a drug which must be respected because of its toxic potentialities but these have been sufficiently well studied that the drug may be used with confidence when therapeutic indications are clear-cut. While doses in excess of 20 mg. per kg. of body weight per day are tolerated very well for short periods of time, less than two weeks in duration, such doses are of considerable toxicity when treatment is continued for several weeks or longer. Doses as low as 10 mg. per kg. of body weight per day rarely display any toxic manifestations. Furthermore, doses of this order of magnitude undoubtedly possess some therapeutic potential but almost surely these smaller doses are not as effective as those of 20 mg. per kg. of body weight per day. For patients of average weight (50 to 70 kg.) this means that the average daily dose which is proven to be effective and of moderate toxicity would be 1.0 gm. per day. If avoidance of toxicity is of paramount importance, doses as low as 0.5 gm. per day may be chosen; but if used it should be recognized that there will likely be some sacrifice of therapeutic efficacy. When the clinical situation is desperate (as in miliary tuberculosis and tuberculous meningitis), the chosen dose will likely be 2.0 gm. per day for a patient of average weight. Such a dose will very likely result in temporary or permanent disturbance of the vestibular functions of the eighth cranial nerve in a fair majority of patients treated with doses of this magnitude for periods of time in excess of thirty days. Even when vestibular function is totally and permanently destroyed, a majority of patients are able to make a satisfactory compensation for loss of such function although compensation may require many months. When the disease being treated is an immediate and serious threat to life and when no alternative treatment is likely to be effective, the physician will not hesitate to assume the risk of such a serious toxic reaction.

Experience has indicated that when the average dose of 1.0 gm. per day of streptomycin is chosen the entire quantity may be administered intramuscularly once each day. There does not appear to be any advantage in dividing the dose either for the avoidance of toxicity or for improving therapeutic results. Modern streptomycin preparations are quite soluble and 1.0 gm. may be readily dissolved in from 2 to 4 cc. of sterile water or saline and injected intramuscularly with no appreciable discomfort.

Dihydrostreptomycin is very similar to streptomycin in most respects. It was developed and has become the most popular form of streptomycin therapy because of the fact that larger doses may be administered with less risk of producing toxicity to the vestibular branch of the eighth cranial nerve. For this reason physicians who prefer larger doses (or when larger doses are required as in tuberculous meningitis and miliary tuberculosis) usually choose dihydrostreptomycin in preference to streptomycin. Under more commonplace conditions when the chosen dose is 1.0 gm. per day many physicians have seen very little if any distinction between streptomycin and dihydrostreptomycin although the margin of safety would appear to be greater when dihydrostreptomycin is chosen. Some earlier lots of dihydrostreptomycin hydrochloride proved to be rather irritating at the site of

intramuscular injection and this has led some physicians to prefer the older drug. Present day preparations are completely devoid of this handicap and are no more irritating than is streptomycin or penicillin, and these preparations may be used not only intramuscularly but also intrathecally without fear of excessive local chemical irritation.

What is scarcely more than a rumor has gained ground to the effect that streptomycin may be clinically more effective than is the newer product, dihydrostreptomycin. Unfortunately there are no well controlled studies which are capable of settling this issue completely but the author has reviewed what data are available, especially those secured by the admirable cooperative study of the U. S. Veterans Administration, the U. S. Army and the U. S. Navy. These data indicate in my opinion that there is no significant difference in therapeutic efficacy between these two drugs. To settle this issue decisively would require an extensive study with absolutely comparable groups of patients selected by an infallible method of randomization treated in identical manners with the two drugs in question, and the results should be analyzed by physicians who do not know which drug was used in each case. Until such studies have been completed, we should regard these two drugs as having the same therapeutic potentiality because all evidence from experiments *in vitro*, *in vivo*, in experimental animals and in such crucial clinical tests as tuberculous meningitis agree that streptomycin and dihydrostreptomycin have similar antituberculosis activity.

Another rumor has gained considerable ground to the effect that dihydrostreptomycin possesses an unusual selective toxic effect upon the auditory function of the eighth cranial nerve. Although it is undoubtedly true that some patients who received dihydrostreptomycin in very large amounts early in the course of investigation with this drug did suffer impairment of hearing, it is now well established that these preparations of dihydrostreptomycin are quite unlike the product which is now commercially available. It was recognized in 1945 when the first patients were treated with streptomycin for prolonged periods of time that this drug would occasionally produce deafness, especially when given in large amounts for prolonged periods of time and when given by intraspinal injection for the treatment of tuberculous meningitis. It should also be mentioned that meningitis itself may result in marked or complete impairment of hearing regardless of the type of therapy used. There is at present no convincing evidence that either streptomycin or dihydrostreptomycin is more toxic to the auditory function of the eighth nerve than is the other drug.

In some of the very earliest reports regarding streptomycin therapy in tuberculosis it was recognized that the long term cumulative toxic effects were often related to impaired excretion of the drug as a result of imperfect renal function. It must be constantly reiterated that both streptomycin and dihydrostreptomycin are much more dangerous drugs to patients who have impaired renal function although the drugs themselves only very rarely modify renal function.

Other adverse reactions to the streptomycin drugs include hypersensitivity reactions with cutaneous manifestations, and it is frequently found that a patient who is hypersensitive to one of the streptomycin drugs may tolerate the other without difficulty.

Para-aminosalicylic Acid. This drug, more conveniently referred to as PAS, has now received wide acceptance as an important therapeutic aid in the treatment of tuberculosis. There is no longer any serious doubt but that the drug by itself has a helpful degree of antibacterial action when administered under clinical conditions. Its potency appears to be distinctly inferior to that of the streptomycin drugs and this is especially demonstrated by the fact that this drug will rarely if ever suffice to produce a remission in tuberculous meningitis unless it is combined with a streptomycin drug. PAS seems to be of greatest usefulness under

three circumstances: (1) to serve as an auxiliary agent to a streptomycin drug, the two being given simultaneously, (2) for use in treatment of tuberculous infections due to bacilli which have become resistant to streptomycin, and (3) as a substitute for a streptomycin drug, especially when the physician anticipates a great need for streptomycin treatment at some remote future date. An example of the latter use, which is encountered most frequently, is in a situation in which pulmonary resection might be possible several months in the future but in the meantime there is a distinct therapeutic objective to be attained and the physician is most eager to preserve the streptomycin sensitivity of the tubercle bacilli so that a streptomycin drug may be used for protection at the time of pulmonary resection.

Perhaps the best rule for dosage of PAS is to administer all the patient will tolerate, the limiting factor being the gastrointestinal irritation produced by this drug. Most patients will tolerate about 12 gm. daily divided into three or four doses and administered with meals. This dose yields an adequate and reasonably continuous blood level and usually can be tolerated for a period of several months. If 15 to 20 gm. per day are tolerated without producing excessive gastric distress or diarrhea, there is likely an advantage to the larger dose.

PAS is available commercially in several forms both as the free acid and as the sodium salt. I believe that most patients tolerate the sodium salt better than the free acid. The sodium salt may be prepared by adding from 60 to 75 gm. of sodium bicarbonate to each 100 gm. of PAS powder to which water is added to make a volume of 500 cc. This mixture will effervesce very strongly for a prolonged period of time, hence a large container must be used. The resultant solution may be flavored with a few drops of oil of peppermint or oil of wintergreen and each 5 cc. will contain 1.0 gm. of PAS equivalent. Patients are often permitted to experiment with various diluents either taking this mixture in plain water or in concentrated form followed with water; others prefer to mix it with milk or fruit juices. Some patients prefer to take the tablets of PAS, and recently we have found some patients who prefer the tablets of sodium PAS as distributed commercially. Those patients whose complaints following PAS administration consist largely of sour stomach and gaseous indigestion sometimes will tolerate one of the several types of coated granular forms of PAS although this type of drug offers no advantage to those who suffer from intestinal distress and with diarrhea.

When combined with a streptomycin drug the amount of streptomycin or dihydrostreptomycin to be given and the duration of treatment will depend upon the clinical circumstances. When the patient is seriously ill, I have usually preferred to give 1.0 gm. per day of dihydrostreptomycin in combination with 12 gm. per day of PAS. However, when the desire is to preserve streptomycin sensitivity for as long a period as possible and where the therapeutic objective may not be quite so urgent, I have often used the dosage schedule proposed by Colonel Carl Tempel and his associates at Fitzsimons General Army Hospital in Denver, Colorado. This consists of administering 12 gm. per day of PAS and 1.0 gm. of dihydrostreptomycin either two or three times per week. Sometimes it is possible to continue this for several months without producing streptomycin-resistant tubercle bacilli.

Many physicians are coming to the belief that a combination of PAS with a streptomycin drug offers a great therapeutic advantage because of the fact which has now been well demonstrated that drug-resistant bacilli appear more rarely at a much later date and perhaps the degree of drug resistance is of a lower order of magnitude than is observed when these drugs are administered alone or in sequence. There is still, however, the very distinct possibility of producing streptomycin-resistant organisms in patients who receive combined therapy, especially if treatment is continued

for periods longer than three or four months. If it can be demonstrated that this drug combination can be administered for as long as two or three months without any real probability of the appearance of drug-resistant organisms, it would appear that antimicrobial therapy might be available to many persons with tuberculosis who would be denied such treatment today because of this fear of developing resistant strains of tubercle bacilli. Not only might the number of candidates for treatment be doubled or trebled by this therapeutic development but also those who do receive treatment might be treated for appreciably longer periods of time with consequent superior therapeutic results.

Tb 1-698. This is the thiosemicarbazone drug which was developed by Domagk in Germany and which has been used so extensively in that country and is now receiving worldwide distribution. The German physicians who studied this drug so intensively have had very little opportunity to compare its efficacy with that of the streptomycin drugs and with PAS because these other drugs were not available to them in sufficient quantity during times of economic stress in Germany. However, physicians in the United States have now had sufficient experience to feel qualified to rate these drugs in a tentative manner and I believe that most would agree that the thiosemicarbazone drug should be relegated to third place in comparison with the streptomycin drugs and PAS. This does not mean by any means that third place is not an important place because tuberculosis is such a prolonged disease that we often need even more than three drugs to be used in sequence.

In the treatment of animals experimentally infected with tuberculosis this drug is highly effective although inferior to the streptomycin drugs. Unfortunately it is not tolerated well by human beings and much smaller doses must be given than would be anticipated on the basis of experience with animals. Most patients will tolerate 50 mg. of Tbl-698 every twelve hours (100 mg. per day) although it is best to start with only 25 to 50 mg. per day for the first week or two. Many patients will tolerate 200 mg. daily; however, if this is used routinely, the incidence of uncomfortable and even dangerous side effects will be rather high.

Patients receiving Tbl-698 should be observed carefully for leukopenia, to avoid if possible the development of agranulocytosis, because bone marrow damage does result in a small percentage of cases. Mild to moderate anemia also may develop but a normal blood picture returns when treatment is discontinued. Liver damage undoubtedly occurs rather frequently but seems to be limited to a mild and reversible fatty infiltration associated with some functional impairment as revealed by such tests as the bromsulfalein dye retention test. Jaundice is rarely encountered and is, of course, an indication for prompt interruption of treatment.

It is not possible to define the indications for treatment with this drug at this time. It may be useful in treatment of tracheobronchial tuberculosis and tuberculosis of the larynx and intestinal tract when organisms are streptomycin-resistant and when PAS cannot be taken because of idiosyncrasy. The toxicity of Tbl-698 appears to contraindicate its use in most cases of minimal tuberculosis and other benign types of disease. I have used Tb-1 in combination with both dihydrostreptomycin and PAS in treatment of tuberculous meningitis but cannot know what degree of added therapeutic benefit it bestowed.

Promizole and Promin. These drugs of the sulfone series are important historically, having preceded streptomycin and having demonstrated that antibacterial therapy in tuberculosis was feasible. Neither has shown itself to be comparable to either streptomycin or PAS under clinical conditions. Promizole has been utilized by Dr. Edith Lincoln successfully in treatment of miliary tuberculosis and in combination with streptomycin and dihydrostreptomycin in treatment of tuberculous meningitis. These were some of the first studies on combined anti-

bacterial therapy of clinical tuberculosis but we still do not know how promizole may compare with PAS as a companion drug to streptomycin.

Promin has gained great prominence as a successful therapeutic agent in treatment of human leprosy. The logic of trying the drug on leprosy was based on its pronounced beneficial effect upon experimental tuberculosis of guinea pigs since both diseases were caused by acid-fast bacilli. It seems probable that promin is chiefly active in experimental tuberculosis by reason of its ability to break down to produce diaminodiphenylsulfone. This latter drug should be tried in clinical tuberculosis.

Neomycin. When neomycin was first announced, it had been studied only *in vitro* and hence no estimate of its potentialities could properly be made. Unfortunately it has been very disappointing *in vivo* because its toxicity for animals was such that they scarcely could be kept alive long enough for its therapeutic benefits to be realized. During the past two years a vast amount of effort by excellent laboratories has failed to produce a neomycin sufficiently free of toxicity to offer hope that it can be helpful in treating human tuberculosis.

Terramycin and Viomycin. These relative newcomers into the field of antibiotics have not been completely investigated at this time. Terramycin is of such a low order of potency against Mycobacterium tuberculosis *in vitro* and in experimental animals that it bears little promise. However, it is a drug of low toxic potential, at least for shorter periods of treatment, and it might yet develop into a useful remedy for mild long term antibacterial effect.

Viomycin has a very narrow antibacterial spectrum being apparently remarkably specific against the bacillus of tuberculosis. However, it does not equal the streptomycin drugs on a weight basis and seems to have some deleterious toxic effects when given for prolonged periods. Some reason remains to hope that viomycin may be purified or improved sufficiently to overcome its apparent toxicity. If this is accomplished, it might assume a useful role in treatment of tuberculosis.

COMMENTS AND SUMMARY

Pulmonary tuberculosis of man responds to several forms of treatment and fortunately some of these may be used simultaneously to achieve the greatest gains. Essentially all patients require prolonged bed rest, many require collapse therapy and a few may qualify for pulmonary resection. Antimicrobial therapy rarely substitutes for these but frequently supports and augments the therapeutic effectiveness of the older therapeutic procedures.

Antimicrobial therapy and other procedures have made the task of the physician and surgeon more responsible and more difficult, and have prolonged the lives of many incurable patients increasing the need for beds and increasing the cost of caring for the tuberculosis problem in any community.

The greatest antimicrobial therapeutic effectiveness with least undesirable side effects will be obtained when moderate doses of dihydrostreptomycin sulfate are combined with maximal tolerated doses of para-aminosalicylic acid. However, the timing of therapy and the choice of therapeutic regimen often require the exercise of such skill and judgment as may be obtained only after much experience with tuberculosis.

Clinical research with such drugs as the sulfones, the thiosemicarbazones and the recently discovered antibiotics (viomycin, terramycin) has not progressed to such a point that these drugs can be recommended for clinical use.

Fundamental Principles of Treatment of Tuberculosis, Including the Use of Antibiotics*

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SUCCESSFUL therapy of tuberculosis implies reversal or repair of the physiologic and anatomic changes resulting from the infection. This requires identification of such changes if rational therapy is to be applied. Therefore, an effort is made to consider treatment of tuberculosis as it may be based on concepts of pathogenesis of the disease and to evaluate various forms of treatment as they may modify its course.

Certain generalizations may be made concerning the patient presenting himself for treatment of tuberculosis. By the time the inflammatory reaction in the lung has become clinically detectable some of the original small areas of inflammation have undergone necrosis and sloughed their contents into adjacent tissues. New lesions result which are of sufficient size for roentgenologic identification. Since necrosis of tissue and bronchial extension have occurred, it is evident that the patient's defenses are not adequate at this point to control the infection. Whatever factors contribute to the rather nebulous total of resistance must be assumed inadequate to arrest the multiplication of bacteria and necrosis of tissue. Later, should sufficient resistance develop to halt this progression of disease, considerable repair of damaged tissue must be accomplished. Otherwise the stage remains set for further sloughing and dissemination of infection which may occur early or late in the course of the disease. While the size of the lesions is of prognostic importance the eventual outcome is more dependent upon their pathologic character. These assumptions apply not only to advanced stages but also to early asymptomatic disease. Persistent unsloughed necrotic areas may serve as a source for future extension of infection. These lesions may organize eventually but the process is slow and often incomplete, leaving areas in which organisms persist. Under these circumstances potentialities remain threatening although the patient is free of symptoms and radiologically evident change in the lesion is slight. Such lesions are probably not truly walled off from the body but remain in physiologic relationship with the surrounding tissues. At present, knowledge is incomplete regarding the chemical and other changes which occur to convert inspissated caseous lesions into sloughing liquid medium containing rapidly multiplying bacteria. This remains an unpredictable hazard and the chronicity of tuberculosis is attributable in large degree to inability of the body to eradicate such lesions. The outcome of therapeutic efforts in any case may depend largely upon the extent of caseation necrosis and the effectiveness with which it is handled. In addition to local extensions of disease within the lung, access to the blood stream through involvement of pulmonary veins or lymphatics may have established foci in distant parts of the body. These may undergo considerable progression before they become clinically manifest but, like early pulmonary disease, respond best when treated early.

With these concepts of pathogenesis in mind, the clinical problem becomes ap-

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parent. First and most urgent, progression of the infection must be halted. This implies suppression of bacterial multiplication and prevention of further dissemination of infection through the bronchi. Resolution of exudate must occur in pneumonic lesions and within cavity walls. Necrotic lesions may have sloughed their contents and persist as cavities in the walls of which tubercle bacilli proliferate with easy access to communicating bronchi. These cavities must be closed for permanent control of the disease. It is likely that caseous foci of some extent persist in many individuals who have made good clinical recovery and will remain well under favorable circumstances, but adequate healing is a long term process. The therapeutic problem may then be summarized as follows: prevention of further dissemination of infection, resolution of exudative lesions, control of caseous residues, closure of cavities which have resulted from sloughing caseous lesions and rehabilitation to a regimen modified to maintain a favorable balance between resistance and residual chronic infection.

It becomes evident that early diagnosis and early effective treatment must be considered an important part of the treatment program. Caseous lesions, as has been implied, are unsatisfactory to treat and constitute a major problem in the control of the disease. They are more effectively avoided than treated. Since progressive disease evolves from a stage in which only minimal caseation has occurred, it appears logical to control it at this point. Failure to do so permits progressive caseation necrosis, spreading disease with further necrosis, thus increasing the therapeutic problem. If early disease is viewed in this way as the potential source of chronic disability, its size becomes therapeutically unimportant except in being more easily controlled than the more extensive lesions into which it may evolve. That early lesions of microscopic size may heal spontaneously is supported by the high incidence of tuberculin conversion without development of progressive disease. It is also a frequent clinical experience to encounter lesions which appear to have been controlled without treatment. Unfortunately, it is not possible except in retrospect to distinguish individuals in whom development of resistance will prove less effective. It is also significant that in many of these cases eventual extension of disease occurs from unpredictable liquefaction of caseous residues. In such instances there seems to be little question that early effective treatment might have avoided late crippling disease.

That some individuals heal tuberculosis effectively while others develop progressive disease emphasizes the necessity for individualization of management. Certain facts are known about each case of tuberculosis at the time of its identification but these apply mainly to the extent and character of the disease process. At this point little may be assumed with safety regarding the ability of the patient to develop resistance. Some contributory influences may be identified and included in the working plan. Age, race and sex are apparently related to the state of resistance. Nutrition and general health also play a part. These influences can be evaluated and are useful in estimating the patient's assets for fighting his disease. Our knowledge is incomplete concerning the character of tissue and humoral response to bacteria and the influence of hormone activity on the course of the disease. We are also unable to predict such unhappy accidents as invasion of unobliterated pulmonary veins with consequent blood stream dissemination or ulceration followed by pulmonary hemorrhage. Dosage of infecting organisms is usually unknown in human disease. Such gaps as these in our knowledge leave the clinician in the unhappy situation of attempting to influence favorably a relationship between bacterial activity and tissue response without knowing the exact nature of the relationship. Under these circumstances therapy tends to be empirical. It remains necessary at many points to rely on accumulated clinical experience, but results are more satisfactory and exact knowledge increases if this is done as

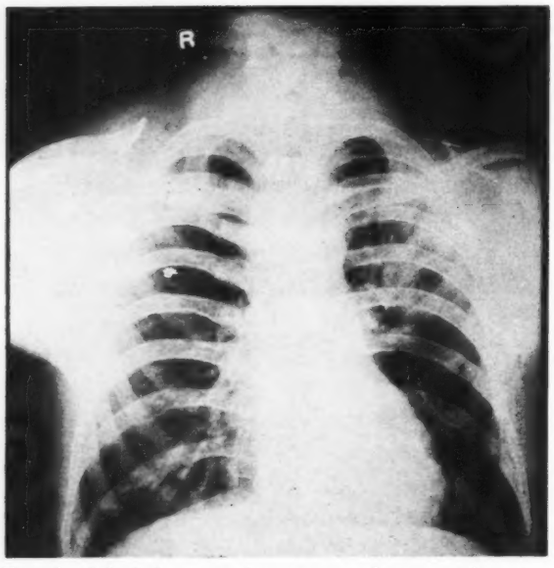

FIG. 1. Admission roentgenogram of twenty-six year old white male.

critically as possible. It is valuable, therefore, to survey various forms of treatment in an effort to identify effective mechanisms and to determine their reasonable point of application in the treatment program. In doing so it must be borne in mind that there is as yet no form of therapy which directly cures tuberculosis. Chemotherapeutic agents now available are bacteriostatic and thus suppressive for varying lengths of time. Collapse therapy approximates walls of cavities and reduces motion of inflamed areas. Surgical resection removes localized segments of diseased tissues. However, the actual process of resolution of exudate and healing of damaged tissues is a reflection of developing tissue resistance without which lasting clinical recovery cannot occur.

It has long been known that rest favorably influences recovery from tuberculosis. While it would be desirable to know the exact manner in which this is effected such information is not available and application of rest therapy depends largely upon the dictates of clinical experience. It is, however, possible to identify certain manifestations of the effects of rest which can be applied clinically. For this purpose the following case is cited in brief summary of pertinent observations (Figs. 1 and 2):

Fever and tachycardia subsided during the first week of bed rest. As is frequently observed the volume of sputum was reduced during this same period and cough decreased in frequency and severity. These observations may be correlated with what is known of the pathologic process. It seems reasonable to postulate that a small area of tuberculous pneumonia had progressed to caseation. Sloughing of the contents of this area had ejected pus containing tubercle bacilli into draining bronchi. Adjacent inflamed bronchi contributed bronchial exudate and secretions. Some of this material had been aspirated into healthy lung, establishing acute tuberculous pneumonia, while the remainder was expectorated. Fever and tachycardia were systemic reflections of absorption of toxic substances from inflamed lung tissues. Their reduction during bed rest may then be due to decreased absorption.

Of major importance is the reduction in volume of bronchial discharges. As previously stated, the first objective of treatment must be to check further dissemination of the infection through the bronchi. The source of the infectious exudate is the sloughing caseous area or cavity which must be assumed to be present although of insufficient size to alter physical signs or to be roentgenologically demonstrable. The quantity and viscosity of necrotic material produced from a cavity are important. As shown by Brown and Archibald in 1927, viscous material is more readily removed than fluid secretions which may flow freely by gravity or be sprayed into healthy lung during inspiration following forced expiration of cough. It becomes important, therefore, to limit in all possible ways the addition of fluids to the pus which must inevitably be evacuated from the cavities present.

The patient at rest is somewhat protected from much of the irritation to respiratory mucous membranes which results from changing temperatures, inhalation of irritating substances and intercurrent infections. This probably contributes to reduction of secretions and further reduction occurs as the inflammatory reaction subsides due to

the general effects of rest. The observation that sputum is reduced when the patient is at rest is of great usefulness and must be applied if the hazard of spreading disease is to be minimized.

Useful information may be obtained from the character and volume of sputum. If the amount of purulent material is large, considerable necrosis and liquefaction of tissue must be assumed. If the amount of watery mucoid sputum is great, irritation of larynx or bronchus is suggested. From the aforementioned may be derived implications for clinical handling of the patient who is producing tuberculous sputum. Bed rest is advisable to reduce the amount of sputum and thus diminish the likelihood of further extension of disease. Inflammatory lesions in bronchi and larynx should be identified and controlled to avoid outpouring of fluid. The serious potentialities of hemorrhage as an additional means of dispersion of infection must be anticipated and avoided when possible. The inexperienced patient must learn that controlled cough is a useful mechanism for removal of discharges from the bronchial tree. He must be taught to inhibit useless forceful cough which is exhausting and hazardous since it is frequently followed by forced inspiration. He will usually learn that cough is reduced when he positions himself with his diseased lung dependent. Explanation of this mechanism will suggest to him the wisdom of sleeping in this position but should also suggest the necessity for reversing his position at intervals to drain dependent bronchi. Restraint in the use of cough-repressing drugs is important. Symptomatic relief can be afforded without depressing the cough reflex to the point of retention of exudate and secretions. Analysis of the character of sputum and factors precipitating cough will often suggest suitable measures.

If the rest regimen is successful, progression of disease is halted and resolution of exudate usually follows in areas in which irreversible tissue damage has not occurred. Resolution returns the tissues to an essentially normal state and is, therefore, the most desirable form of healing. This emphasizes the importance of early intensive rest before permanent damage has taken place. In such cases it may be expected to decrease manifestations of toxemia, diminish the danger of disseminating infection and establish

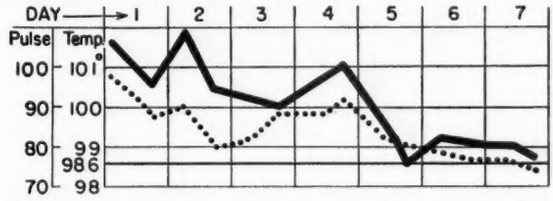

FIG. 2. Temperature and pulse record during the first week of bed rest.

circumstances favorable for resolution. Some degree of exudative reaction is present in most chronic active lesions and may be expected to respond favorably in similar manner. The instability of exudative lesions makes close observation necessary if unfavorable changes are to be identified and treated effectively. For this reason bed rest is best administered in a hospital. Details of the rest program must be worked out with the patient whose full understanding and cooperation are essential. This usually requires day-to-day discussion, adjustments and encouragement.

It is of greatest importance at the institution of treatment to attempt to evaluate the situation for the patient in terms of a long range program and eventual return to his normal or modified regimen. This requires careful evaluation of the pathologic potentialities of his disease, translated into probable length and type of therapy. But because the patient's resistance can be estimated only in part and because it is frequently difficult to determine the extent of irreversible damage, such prognostications should be made only in general terms.

A long term of inactivity with some necessary uncertainty as to treatment and outcome is emotionally difficult but tolerated reasonably well by most patients. Their confidence and cooperation being essential to a favorable outcome, it is important to recognize various ways in which thoughtful clinical handling can minimize emotional

problems. Early unwarranted specific commitments regarding the length of time necessary for cure lead to disappointment and loss of confidence even though a favorable course may be in progress. Because the period of treatment is long, a patient may encounter numerous different physicians during this period. Failure to maintain a consistent program results in insecurity for the patient. This is avoidable by careful exchange of ideas among those contributing to clinical management. The patient's personal, economic and social problems must be identified and met if adequate rest is to be achieved. The cooperative efforts of nursing staff, social service worker and vocational guidance counselor are desirable.

In many cases the addition of bacteriostatic drugs hastens the subsidence of toxemia, decreases the hazard of disseminating disease and encourages the process of resolution. Streptomycin and para-aminosalicylic acid are the drugs most widely used in the treatment of tuberculosis. They have proved to be of benefit in certain situations when used singly or in combination, but their usefulness is limited by specific characteristics of the pathologic process in tuberculosis and by the occurrence of bacterial resistance. Therefore, it is desirable to visualize the nature of the pathologic changes and the beneficial effect which might be expected from suppressing bacterial activity for a limited period of time. Choice of the bacteriostatic agent and duration of treatment will depend upon estimation of the patient's native resistance to the disease as well as appraisal of the extent of tissue necrosis which has already taken place. Ideally, the chemotherapeutic regimen selected should be integrated with a tentative program for the long term management of the case, realizing that in many instances the plan may be modified repeatedly as one or another of the pretreatment estimates proves to be mistaken.

The effect of streptomycin in different types of cases is now reasonably well established. Its bacteriostatic action is most pronounced and is accompanied with rapid symptomatic improvement in predominantly exudative tuberculosis in which the inflammatory reaction is acute. Here one may expect prompt subsidence of fever and other symptoms of toxemia, and appreciable decrease in cough and sputum. When laryngeal or endobronchial tuberculosis is present, the rapid diminution in fluid secretions is noteworthy, as is the subsidence of laryngeal pain. Symptomatic improvement occurs concomitantly with decrease in edema and other evidence of inflammation.

When inflammatory changes are less acute, objective evidence of response may be delayed although the patient experiences subjective improvement. It is a frequent occurrence to see little roentgenologic change during a four- or six-week course of streptomycin only to observe marked clearing two to four months after conclusion of treatment. Resolution may lag even in acute cases, and this adds to the difficulty of determining duration of treatment on the basis of therapeutic response.

Since the action of streptomycin is bacteriostatic, its effect is most marked when the preponderance of bacterial activity is in potentially reversible lesions which are susceptible of penetration by the drug. The evidence available at this time suggests that there is little effective penetration of caseous lesions and there is no reason to believe that scarred, fibrous areas are affected by bacteriostatic agents. However, it must be acknowledged that clinical differentiation of the exudative and caseous elements in pulmonary lesions may be difficult except in retrospect. While caseous tuberculosis may be little affected by streptomycin some clinical improvement is noted in most cases. As a rule there is decrease in fever and in other symptoms of toxemia although the response may be rather slight and slow in comparison with the improvement in exudative disease. In so-called caseous pneumonia this partial response may be related to bacteriostasis in the exudative components which are almost always present and may be correlated with decrease in the density or homogeneity of consolidation as

observed roentgenologically. Further, there may be some bacteriostasis in caseous areas, at least in the periphery, although evidence on this point is still uncertain.

Perhaps the most striking effect of streptomycin in extensively caseous pneumonia is the prevention of spread of the tuberculous process. Dissemination to previously unaffected areas in the lungs is rare unless bacterial resistance to streptomycin has developed, but sloughing of necrotic foci may occur and cavities appear during treatment. In such cases sputum may be copious, purulent and heavily laden with bacilli. Spread of the disease through the bronchi and implantation of new areas might be expected under these conditions if it were not for the suppressive effect of streptomycin. When this effect is removed through discontinuing treatment or through the development of significant bacterial resistance, relapse or spread is apt to occur.

The bacteriostatic effect of para-aminosalicylic acid alone is considerably less than that of streptomycin, but bacterial resistance to it usually does not occur in less than four to six months. Under its influence there may be appreciable reduction in cough and sputum, thus decreasing the likelihood of spread through the bronchi. If there is tuberculous inflammation of the larynx or a wheeze related to edema of a bronchus, these signs may begin to clear within a week or two. Resolution of exudative tuberculosis in the lungs may occur under the influence of para-aminosalicylic acid although the response is less prompt than with streptomycin. Its effect has perhaps been most clearly demonstrated in patients whose disease has relapsed on bed rest following cessation of streptomycin therapy. In some cases the relatively mild suppression of bacterial activity by para-aminosalicylic acid when continued for a number of months has been sufficient to allow resolution of extensive disease.

While the usefulness of para-aminosalicylic acid in certain situations has been established, streptomycin remains the most potent bacteriostatic agent against Mycobacterium tuberculosis yet available. During the last four years widespread efforts have been made to find a regimen of treatment which would provide sufficient bacteriostasis in most cases, yet avoid the development of bacterial resistance. Significant bacterial resistance to streptomycin has been found in approximately 70 per cent of the patients treated for ninety to 120 days and in approximately 20 per cent of the patients treated for twenty-eight to forty-five days. Since the incidence of resistance rises as treatment with streptomycin is continued, various regimens have been investigated in different institutions. From these studies it has become apparent that the emergence of bacterial resistance is related to the type of tuberculosis as well as to the duration of treatment. Resistant organisms appear relatively early in chronic cavitary disease and little benefit can be expected from a few weeks of treatment in this type of case. However, the greatest need for continued bacteriostasis occurs in patients with poor native resistance to tuberculosis who have extensively caseous disease with active sloughing of the necrotic areas. It is in this situation that significant bacterial resistance is apt to occur early. Even when treatment with streptomycin is limited to twenty-eight days, the bacterial population frequently will be predominantly resistant at the end of treatment or shortly thereafter. Relapse within a month or two is common and further streptomycin will not influence the course of the disease under these circumstances. Some means of prolonging the period of effective bacteriostasis is essential if progressive disease is to be avoided in this group of patients.

The use of para-aminosalicylic acid as an adjuvant to streptomycin is promising, for emergence of bacterial resistance to streptomycin appears to be deferred appreciably when these two bacteriostatic agents are used together. However, adequate information is not yet available as to the rate of development of resistance in relation to the nature of the pathologic changes when this type of combined therapy is used. Until

there is more evidence on this point it would seem reasonable to limit the duration of treatment with streptomycin to the minimum believed necessary in a given case. A lesser degree of suppression of bacterial activity may be maintained for several months by continuing para-aminosalicylic acid alone.

As indicated earlier the choice of regimen of treatment depends upon the extent and presumed pathologic characteristics of the disease and upon visualization of the plan of treatment as a whole. In certain instances of advanced bilateral disease it may be justified to give streptomycin and para-aminosalicylic acid for many months. The findings of D'Esopo and Medlar in studying the bacteriologic and clinical effects of prolonged treatment with these two agents are especially interesting and stimulating. Treating almost hopelessly advanced cases, they have shown that effective suppression of bacterial activity may be maintained in many instances for from six to twelve or more months. Consistently negative cultures have been obtained even in the presence of open cavities; but if treatment with the drugs is discontinued after six months, cultures again may be positive and then will be resistant to streptomycin. It follows that if prolonged therapy of this type is used, treatment must be continued until residual lesions are well controlled, with or without surgery. Until more information is available it would seem desirable to consider long-continued treatment with streptomycin and para-aminosalicylic acid as still in the experimental phase.

It is well to remember that the effect of bacteriostatic drugs is primarily to suppress bacterial activity and that resolution of disease resulting from their use is in a sense artificial, occurring as it does without relation to the patient's native resistance. The rate and possibly the degree of resolution of exudative tuberculosis is increased, and necrosis of exudative lesions is minimized. However, relapse following withdrawal or loss of effect of the bacteriostatic agents is frequent in patients with poor vital resistance to tuberculosis. The likelihood of relapse will depend upon the character and extent of the residual lesions. These should be identified and evaluated as accurately as possible with respect to their potentialities as a source of future dissemination of the disease. Since cavities may disappear during treatment as a result of marked resolution of the surrounding inflammatory reaction and are then difficult to localize roentgenologically, every effort should be made to determine the exact location of lesions before treatment is begun. Decision as to the need for collapse therapy or surgical excision will depend upon critical evaluation of the response to therapy and the character of the residual lesions in relation to the patient's estimated resistance to the disease.

The application of surgical treatment to pulmonary tuberculosis has been extended during recent years by the development of operative technics, improvements in anesthesia and the availability of effective chemotherapeutic agents. Long term results of new procedures are not yet known and caution must be observed in their general application. Procedures currently in use include collapse of lung parenchyma with pneumothorax, pneumoperitoneum, phrenic paralysis or thoracoplasty; excision of lung or pleura and drainage of pulmonary cavities or pleural space. Surgical treatment is concerned essentially with problems resulting from caseation necrosis and excavation of these necrotic foci. Therefore, it is primarily the treatment of progressive forms of the disease.

The effects of collapse therapy are not completely understood. The lung is permitted to relax by its own elasticity and is thereby reduced in volume. Physiologic activity is decreased as evidenced by reduction in ventilation, vital capacity and oxygen uptake. There is decrease in pulmonary blood and lymph flow. It is interesting to note that these observations also have been noted in varying degree during bed rest. In the collapsed lung the elastic stretch and the pull on cavity walls is decreased, allowing them to become smaller and per-

mitting coaptation of the walls. The mechanical features of collapse do not set all of the conditions necessary for control of tuberculous lesions. Its effectiveness is probably due to combination of mechanical and physiologic effects together with increased general resistance to the disease.

Pneumothorax, phrenic paralysis and pneumoperitoneum are reversible procedures. Each has enjoyed a period of enthusiasm modified in time by increasing experience and familiarity with complications which have resulted. The frequency of pleural complications with pneumothorax and the decreasing risk from thoracoplasty have shifted the choice toward the irreversible procedure. While pneumoperitoneum avoids pleural complications its exact field of usefulness remains to be evaluated. Pneumoperitoneum and phrenic paralysis exhibit the mechanical and physiologic effects of collapse therapy previously described but to a lesser degree than do pneumothorax or thoracoplasty. Because they afford symptomatic improvement one is tempted to continue them after indications for more definitive treatment become apparent. Recognition should also be given the importance of the diaphragm in the expulsive mechanism of the lung. Its paralysis may contribute to retention of discharges and consequent spread of disease through the bronchi. Failure of return of function of the diaphragm may limit ventilatory function sufficiently to compromise subsequent definitive therapy.

Tuberculous lesions are usually close to the pleura. Separation of visceral and parietal pleura decreases protective formation of adhesions which tend to localize infection. In the presence of acute disease pneumothorax is often complicated by empyema which may be more difficult to control than the original parenchymal lesions. For this reason pneumothorax may well be deferred until the acute reaction has subsided.

The frequency of pleural complications during therapeutic pneumothorax has made it apparent that the procedure should be abandoned promptly when not accomplishing its purpose. Many difficulties from pneumothorax have resulted from maintaining poor collapse in preference to other forms of treatment.

At times the severing of adhesions by intrapleural pneumonolysis may improve an ineffectual pneumothorax. The feasibility of this procedure often must be determined by thoracoscopy. Consideration must be given to the character and extent of the adhesions, their proximity to tuberculous lesions and blood vessels. Much of the nutrient blood supply to the walls of cavities may be carried from the chest wall by vessels within the adhesions. Interruption of this supply may interfere with nutrition sufficiently to cause necrosis of the wall. In other instances a diseased upper lobe may be suspended from the thoracic cage in such manner that release of adhesions permits the lobe to assume a position unfavorable for drainage of discharges. When performed judiciously pneumonolysis is a useful procedure but when hazardous or of questionable benefit it is preferable to abandon pneumothorax.

Thoracoplasty is a procedure of established value by which the majority of upper lobe lesions may be effectively controlled. Thoracoplasty decreases the size of the thoracic cage and limits motion of the collapsed areas. This permits relaxation of lung tissue and approximation of cavity walls. It is also useful at times in obliterating empyema spaces. Since it is usually applied over areas of damaged tissue in which gas exchange is minimal, the resultant further reduction of pulmonary function in this area is slight. The degree of collapse is, however, usually more extensive than the diseased area. For this reason consideration should be given to anticipated alterations in function since the procedure is irreversible.

During thoracoplasty discharges are poorly eliminated from the respiratory tract and there may be dissemination of disease if the operation is performed when secretions are excessive. Immediately following operation cough is ineffectual due to paradoxic

motion of the operated chest wall. Motion is painful and the cough-depressing effect of many analgesic drugs favors retention of secretions. This hazard is reduced by careful attention to posture and drainage.

Resection of pulmonary tissue is being used increasingly and indications are being clarified. Excision is now accepted generally as the treatment of choice for lower lobe cavities, progressive disease under thoracoplasty, tuberculous bronchostenosis, extensive destruction of one lung, and tuberculosis and suppurative disease within the same lobe. Resection of segments of lobes is a recent procedure designed to remove potential sources of dissemination with maximum economy of healthy lung tissue. Pathologic study of surgical specimens frequently reveals lesions of insufficient size for radiologic demonstration. Their presence should be assumed in the lung remaining after excisional therapy.

Tuberculous pleural effusions usually subside and resorb within six to eight weeks unless pneumothorax is present. The separation of pleural surfaces by gas reduces the amount of absorbing membrane in contact with fluid and prevents the apposition of membranes which might otherwise adhere and obliterate the infected space. Occasionally, simple effusions persist and become purulent, but most tuberculous empyema is encountered as a complication of pneumothorax. Treatment of empyema requires control of infection and elimination of the space occupied by the fluid and perpetuated by organization of the visceral pleura. In the past this has required extensive thoracoplasty with removal of the parietal pleura. This procedure was poorly tolerated by patients who were debilitated by chronic infection and loss of body protein through drainage from the infected pleura. Recently it has become possible to remove the restricting membrane by decortication. The infected space is thus obliterated by re-expansion of lung. Considerable pulmonary function may be restored by this means if the re-expanded lung is physiologically healthy. In some cases organization resulting from parenchymal or pleural disease may limit re-expansion. In this situation thoracoplasty or removal of the involved lung and pleura may be necessary.

For satisfactory results it is of greatest importance that surgical therapy be integrated into the long term program. This program is best devised by combined medical and surgical consultation early in the course of treatment. While surgical treatment is the effective means of controlling certain phases, it is not curative of the systemic infection which is usually present. Long term treatment remains a necessity although localized manifestations of the disease are well controlled by surgical measures.

The therapeutic regimen may be summarized as follows: From bed rest may be anticipated prevention of dissemination of disease through the bronchi, diminution of systemic evidences of toxemia, circumstances favorable for resolution of exudative disease and increasing general resistance to tuberculosis. Chemotherapy through suppression of bacterial activity has many of the same effects but they occur more promptly. Collapse therapy permits cavity closure and reduces physiologic activity of the lung. Surgical excision removes permanently damaged tissues. Through combinations of these methods control of acute disease and repair of resultant anatomic defects are effected. The probability of remaining small foci of caseation must be assumed although cavities are no longer evident by roentgenography and absence of tubercle bacilli from sputum confirms their control. It is hoped that over a period of time this residual disease will be completely healed. To this end a careful program of supervised convalescence and rehabilitation must be planned. Vocational changes and economic assistance may be necessary. The patient should have learned much concerning his disease and should have accepted the necessity for slowly graduated activity, necessary concessions to his disability and periodic supervision.

Changing Pattern of Tuberculosis Control*

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If we were to take the globe of the world and shade the various areas according to the seriousness of the tuberculosis problem within each area, the resulting visual impression would be that of a mottled, almost pock-marked world. Amid vast expanses of black we would see an occasional island of white and perhaps more frequent gray mottlings.

Asia and Africa, for example, would comprise vast seas of black, with estimated death rates running between 260 and 360 per 100,000 for the former and between 240 and 300 for the latter.[1] Europe would be largely gray, with occasional patches of white in such countries as Denmark (with the world's lowest death rate),[2] the Netherlands and England and Wales; for the most part rates in the European continent would fall between 110 and 130.[1] In the western hemisphere, too, the visual impression would once again be largely gray, with occasional dark areas such as Brazil, Chile[3] and Alaska, and the white areas confined largely to North America, in Canada and the United States.

In the aggregate it is estimated that tuberculosis accounts for four to five million deaths each year the world over. Moreover, it represents the chief cause of death among children throughout the world according to the United Nations International Children's Emergency Fund.[4]

These are some of the varying situations with which the world has to contend in the fight against tuberculosis, and generally, more refined definition of such data gives direction to tuberculosis control efforts in each area. Because the factors are different from one area to another, the direction of control efforts will of necessity be different. These are the considerations, among others, which have governed and will continue to govern the intensity and vigor as well as the direction of our own control program here in the United States.

Since the turn of the century we have seen many marked changes in public health within the United States. We have seen the growth and development of a public health network which today offers a wide variety of full time services to more than seven of every ten men, women and children throughout the nation.[5] Whereas fifty years ago we concerned ourselves with sanitation and the provision of safe drinking water, today we go beyond this and devote our time and energies to specific programs for the prevention of disease as well. Indeed, the emphasis now is on general health, with broad programs involving heart disease and cancer control, maternal and child health, mental health and the control of communicable diseases. The general death rate has fallen from more than 1,700 per 100,000 to 990 in the last half century, life expectancy at birth has risen from less than fifty to more than sixty-seven, and the entire composition and pattern of the leading causes of death has changed so radically that today it bears little resemblance to that which existed in the early part of the century.[6–8]

All these changes in the general health level of the nation, as well as the changes which have occurred in the pattern of tuberculosis itself, have a most direct bearing on the course and direction of our present tuberculosis control efforts. Indeed, if the changes in general health have been great in the last half-century, they have been no less extensive for tuberculosis itself.

Fifty years ago almost 200 of every

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100,000 Americans died of tuberculosis. By 1948, however, the rate had plummeted downward more than 80 per cent to 30 per 100,000 and is still coming down. The first cause of death for the population as a whole in 1900, tuberculosis is now in seventh place among the leading causes of death (Table 1), having been displaced by such diseases as cancer and heart disease. The picture of tuberculosis control has improved vastly, too, within our several states. As may be seen in Figure 1, every state in the union experienced a considerable reduction in tuberculosis mortality rates, even in the brief period of eleven years between 1938 and 1948. Moreover, using the quartile distribution of 1938 for both 1938 and 1948, the two maps in Figure 1 illustrate how every state, with the exception of Arizona, has moved up beyond the limits of its 1938 quartile standing, and by 1948 virtually all the "black" areas in the 1938 map have disappeared.

TABLE 1
LEADING CAUSES OF DEATH FROM 1900–1940 IN ORDER OF RANK

1900	1910	1920	1930	1940
1. *Tuberculosis*	Heart disease	Heart disease	Heart disease	Heart disease
2. Pneumonia	*Tuberculosis*	Pneumonia	Cancer	Cancer
3. Diarrhea	Pneumonia	*Tuberculosis*	Nephritis	Intracranial lesions
4. Heart disease	Diarrhea	Intracranial lesions	Intracranial lesions	Nephritis
5. Intracranial lesions	Intracranial lesions	Nephritis	Pneumonia	Pneumonia
6. Nephritis	Nephritis	Cancer	*Tuberculosis*	Accidents
7. Accidents	Accidents	Influenza	Accidents	*Tuberculosis**

* In 1947 tuberculosis was still in seventh place.
Note: Accidents exclude motor vehicle accidents.

It should be apparent, then, that the progress we have made in the control of tuberculosis in the United States within recent years has been an achievement of no small proportions. Can we now, therefore, look toward practical eradication of the disease in this country? The answer to this question will depend on several things. To a great extent it will depend on the success of our case-finding and the precision and acuity of diagnosis. These are the basic prerequisites to effective control efforts in the United States where, as will be illustrated later, our problem is becoming one of dealing with residual sources of infection.

In years past we believed that tuberculosis was largely a household problem—and there were times when few households did not have a case of tuberculosis; hence we relied largely on routine contact investigation as a case-finding device. This coupled with the examination of suspects (those with apparent symptoms) was an effective approach to the whole question of tuberculosis case-finding. Today, however, we have discovered that such case-finding efforts do not meet our requirements. Even if we were able to do the most exhaustive household contact supervision, we would miss completely the casual contact who if tuberculous would remain free to perpetuate the infection. Moreover, since many tuberculous persons have only vague symptoms, or no symptoms at all, even the most intensive suspect investigation cannot be relied on. Finally, case-finding among contacts and suspects succeeds in bringing to light only a mere handful, some 10 to 20 per cent, of the total number of cases we have reason to believe actually exist in the population.

Today we approach tuberculosis case-finding through the mass chest x-ray examination of apparently healthy persons. In 1949 some fourteen million people in the United States received chest x-ray examinations for tuberculosis in mass screening programs conducted by official and

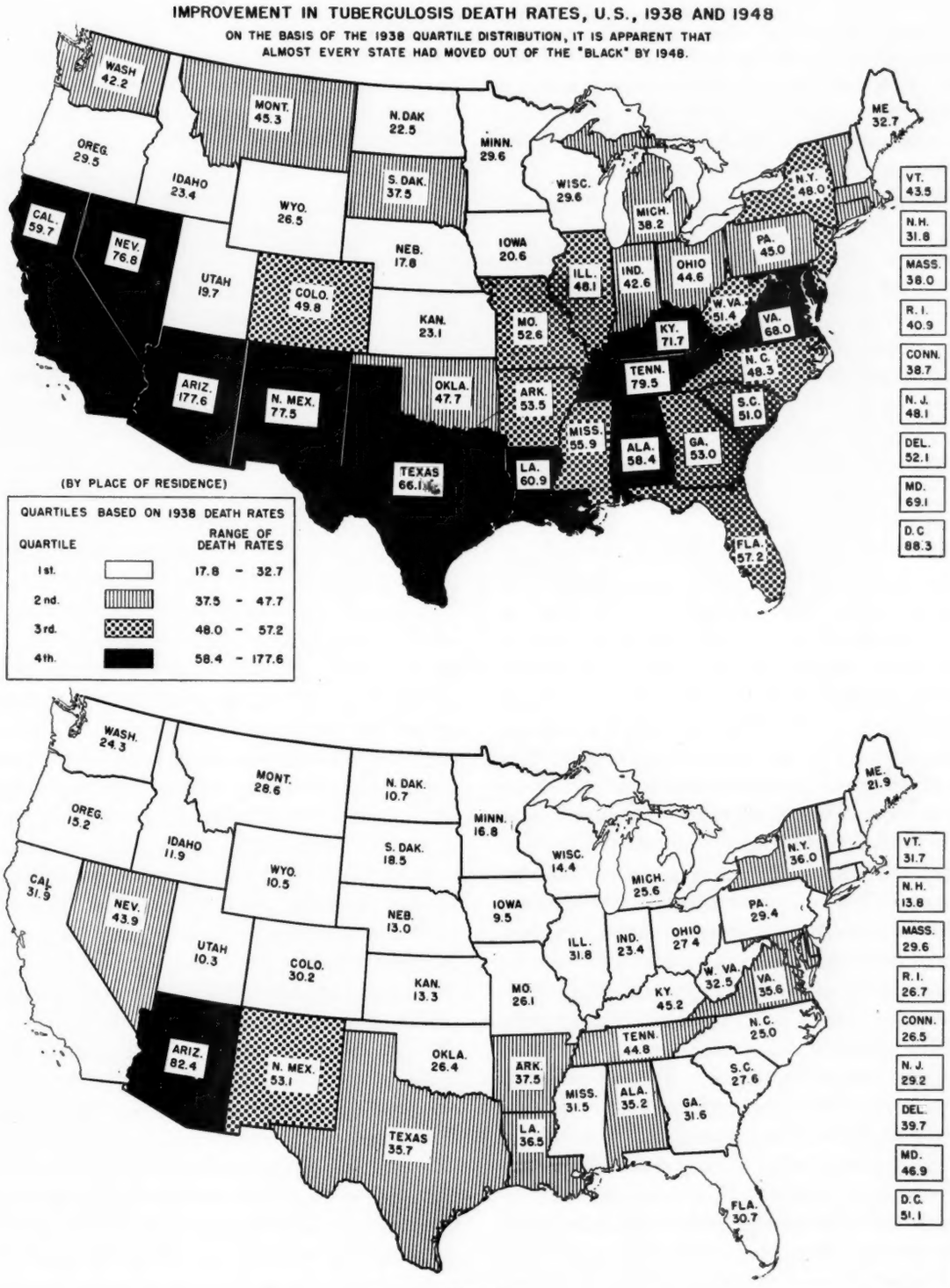
IMPROVEMENT IN TUBERCULOSIS DEATH RATES, U.S., 1938 AND 1948
ON THE BASIS OF THE 1938 QUARTILE DISTRIBUTION, IT IS APPARENT THAT ALMOST EVERY STATE HAD MOVED OUT OF THE "BLACK" BY 1948.
(BY PLACE OF RESIDENCE)
QUARTILES BASED ON 1938 DEATH RATES
QUARTILE
RANGE OF DEATH RATES
1st. 17.8 - 32.7
2nd. 37.5 - 47.7
3rd. 48.0 - 57.2
4th. 58.4 - 177.6
WASH 42.2
OREG. 29.5
CAL. 59.7
NEV. 76.8
IDAHO 23.4
MONT. 45.3
WYO. 26.5
UTAH 19.7
ARIZ. 177.6
COLO. 49.8
N. MEX. 77.5
N. DAK 22.5
S. DAK. 37.5
NEB. 17.8
KAN. 23.1
OKLA. 47.7
TEXAS 66.1
MINN. 29.6
IOWA 20.6
MO. 52.6
ARK. 53.5
LA. 60.9
WISC. 29.6
ILL. 48.1
MICH. 38.2
IND. 42.6
OHIO 44.6
KY. 71.7
TENN. 79.5
MISS. 55.9
ALA. 58.4
GA. 53.0
FLA. 57.2
W. VA. 51.4
VA. 68.0
N. C. 48.3
S. C. 51.0
PA. 45.0
N. Y. 48.0
ME. 32.7
VT. 43.5
N. H. 31.8
MASS. 38.0
R. I. 40.9
CONN. 38.7
N. J. 48.1
DEL. 52.1
MD. 69.1
D. C. 88.3
WASH. 24.3
OREG. 15.2
CAL. 31.9
NEV. 43.9
IDAHO 11.9
MONT. 28.6
WYO. 10.5
UTAH 10.3
ARIZ. 82.4
COLO. 30.2
N. MEX. 53.1
N. DAK. 10.7
S. DAK. 18.5
NEB. 13.0
KAN. 13.3
OKLA. 26.4
TEXAS 35.7
MINN. 16.8
IOWA 9.5
MO. 26.1
ARK. 37.5
LA. 36.5
WISC. 14.4
ILL. 31.8
MICH. 25.6
IND. 23.4
OHIO 27.4
KY. 45.2
TENN. 44.8
MISS. 31.5
ALA. 35.2
GA. 31.6
FLA. 30.7
W. VA. 32.5
VA. 35.6
N. C. 25.0
S. C. 27.6
PA. 29.4
N. Y. 36.0
ME. 21.9
VT. 31.7
N. H. 13.8
MASS. 29.6
R. I. 26.7
CONN. 26.5
N. J. 29.2
DEL. 39.7
MD. 46.9
D. C. 51.1

FIG. 1.

voluntary health agencies throughout the country. In and of itself this is indeed a magnificent achievement and an accomplishment of staggering proportions. As time goes on, however, and more and more active cases of tuberculosis are brought to light as the result of these vast programs, the residual cases will grow increasingly elusive and more difficult to find. Case-finding will then assume greater urgency than ever before in order to prevent the transfer of infection and maturation of new cases and in order to protect the gains already made. It is for this reason that the scope of our case-finding activities will need to be broadened considerably in the future and the intensity of our vigilance strengthened. Only in this fashion will every case of tuberculosis be brought under management at a time when the disease can be dealt with most effectively.

Once the suspects have been screened out of the population, however, complete and accurate differential diagnosis will need to follow promptly in order to bring the active cases of disease under necessary care at the earliest possible moment. This is the second prerequisite to any hope for effective tuberculosis control in the immediate future, and it is at this point that the physician will face a most serious responsibility: the elucidation of an x-ray shadow which, although it may suggest tuberculous activity, may not be tuberculosis at all. From our experience in mass chest x-ray surveys we have learned over and over again that pulmonary x-ray shadows of obscure etiology and significance are quite prevalent among apparently healthy persons. Garland[9] points out that there are more than eighty distinct types of pathologic conditions which are capable of casting chest x-ray shadows. In consequence the physician who must determine the etiology and nature of an x-ray shadow brought to light as the result of a screening examination indeed faces not only a perplexing problem but also a grave responsibility.

Fortunately most cases of tuberculosis found in screening surveys prove to be minimal in involvement. For the patient and his family this is a happy fact. For the physician who must direct the differential diagnosis, however, this fact poses a difficult problem. The typical case of minimal tuberculosis does not present the classical signs and symptoms and is often sputum-negative. Consequently the physician will often find it necessary to piece together scanty clinical, bacteriologic and roentgenographic information. Nor does the presence of a positive tuberculin reaction serve to resolve the problem conclusively.

In an effort to watch for characteristic x-ray changes or more definite bacteriologic findings the physician is obliged to resort to a period of observation before completing the diagnosis. If the diagnostic problem proves to be a typical case of minimal tuberculosis, the evaluation may take as long as ten months or more during which there is, of course, the risk of progression. If it proves to be pulmonary cancer, however, the physician has indeed assumed additional responsibility by deciding to observe the patient long enough to rule out tuberculosis without also including in the diagnostic work-up procedures which might detect the presence of cancer. As Overholt[10] has pointed out, the most readily resectable stage of pulmonary cancer coincides with its "silent phase" so that any delay in diagnosis may be extremely hazardous.

In an area of gross prevalence and heavy mortality from tuberculosis the diagnostic problem is not, in the mass, a difficult one. In such an area chance alone should favor the diagnosis of tuberculosis and certainly public health considerations should make it the preferred diagnosis in doubtful cases. Such a situation might well prevail in a country like India, for example, where there are perhaps two million active cases of tuberculosis and one-half million people die of the disease annually.[4] Furthermore, few people in India live to an age when the suspicion of cancer or heart disease may complicate the interpretation of an x-ray shadow.

In our own country, on the other hand, tuberculosis is not quite so prevalent a

problem. In some areas, particularly in some of our mid-western states, the tuberculosis death rate now stands near ten per 100,000, and tuberculin sensitivity rates are extremely low. It is for just these reasons, as a matter of fact, that the application of such an agent as BCG vaccine can be highly selective and based on individual circumstances of exposure. Indeed, the annual incidence of tuberculous infection appears to be less than 0.5 per cent for our school-age population. Here, then, diagnosis must be especially keen, for a case of tuberculosis missed through faulty or incomplete diagnostic work-up is a serious oversight and one which may later prove costly in terms of personal illness and public health.

Even in those American cities and states where tuberculosis mortality remains relatively high tuberculosis cannot be considered to be the diagnosis of choice in doubtful instances as it can very well be in most parts of Asia or in some of the nations of Europe and South America. In those areas of the United States where tuberculosis mortality is highest the death rates do not match the rates of these other lands. Even so, we cannot justify the facilities misused or misdirected or the personal trauma caused by an incorrect diagnosis of tuberculosis. Moreover, considering the fact that we in the United States are dealing with an aging population, an unsupported diagnosis of tuberculosis may well mask until too late the more lethal disease of cancer, with obviously disastrous results.

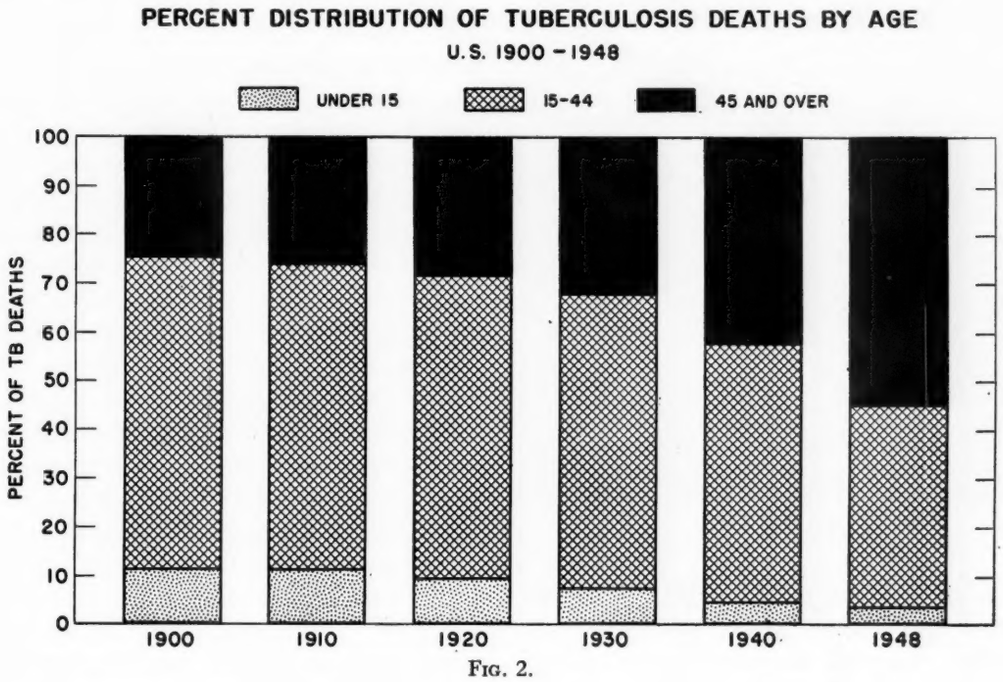

FIG. 2.

In some of our states the physician's difficulties will be compounded further by the extensive prevalence of clinical syndromes which simulate tuberculosis, especially in their roentgenographic appearance. These will include pulmonary shadows associated with histoplasmosis in the central states and with coccidioidomycosis in the southwestern states. In clinical terms these conditions may not in themselves represent any major problem. The associated pulmonary calcifications and shadows, however, will pose a diagnostic problem calling for unusual skill and acuity.

Even in its most frank form tuberculosis may be a difficult disease to diagnose. Only with effort and care can we gather the objective data upon which to base determinations of differentiation, activity, stage of disease and prognosis. To account for x-ray

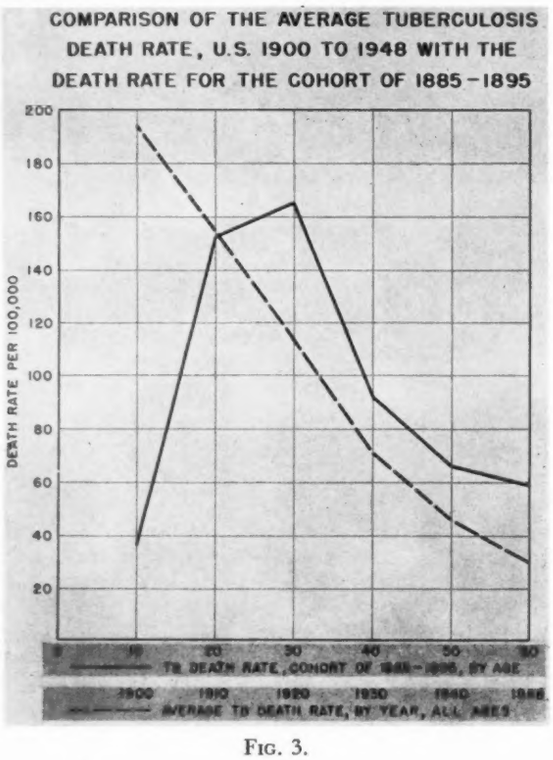

FIG. 3.

shadows of obscure etiology the physician may therefore find it necessary to resort to the entire range of diagnostic procedures, with serial x-ray studies, stereoscopic and laminographic films, skin tests, laboratory and bacteriologic studies, bronchoscopy, biopsy and even surgical exploration of the chest. Only in this fashion can the diagnosis and subsequent management of individual cases be meaningful. And only in this fashion can we hope to gain the most rapid progress toward the control of tuberculosis.

Among certain groups of the population vigilance and acuity will need to be even keener. The vital statisticians tell us that the risk of tuberculosis mortality is more serious among men than women, and mass case-finding programs appear to give support to this observation in terms of prevalence.[11] Moreover, tuberculosis mortality among non-white groups is three times as high as for the white population. Finally, the whole pattern of tuberculosis mortality in terms of age has shifted radically since the beginning of this century so that today it is a far more serious problem among the older age groups than among the younger ones. Whereas in 1900 about 75 per cent of all tuberculosis deaths occurred in those forty-four years of age and younger, 55 per cent of all the deaths now occur in those over forty-five. (Fig. 2.)

For the American population as a whole tuberculosis mortality has fallen sharply and is continuing to drop. For persons sixty-five years of age and over, however, the decline has not been so great. In fact the death rate for white males in this age group was slightly higher in 1948 than in 1939–1941.[12] Epidemiologically there may be sound reason for the shift in the seriousness of the tuberculosis problem to the older age groups. In years past those of our older population (those born at about the same time) lived through periods of great mortality risk, so that the effects of the previous extensive exposure may still be manifesting themselves in residual high mortality among the older age groups. To illustrate, persons who today are sixty years of age have a tuberculosis mortality rate of about sixty per 100,000 population; however, thirty years ago, when they were part of a group thirty years of age, 165 per 100,000 of that group died of tuberculosis. In 1920 the death rate for the thirty year olds was but 46 per cent higher than the rate for all ages; today, however, the much lower rate for this group (now sixty years old) is double that for all ages. (Fig. 3.)

Unusually vigorous efforts are therefore indicated among our older population in order to find the cases of tuberculosis among them and to bring them to treatment at the earliest possible moment. Certainly the concurrently high incidence of cancer and heart disease among this section of the American population should make case-finding and thorough, speedy diagnosis a matter of the greatest concern for all physicians.

It would be interesting to speculate on what our general picture of tuberculosis control might be like today had we thirty years ago been able to prosecute case-finding, diagnosis and treatment with the

same breadth and vigor that we now know is indicated. Those same persons who are now sixty were then living at a time when thirty year olds were dying of tuberculosis at a very heavy rate. At the same time, many of them were then young parents and had extensive contact with children. Today the children themselves, now about thirty years of age, find tuberculosis their first cause of death. What, then, would the tuberculosis death rate for these children now be and, consequently, the general tuberculosis death rate had we in 1920 had available the kind of tuberculosis control we now have?

In the future we can reasonably expect that case-finding efforts of official and voluntary health agencies will expand progressively until the entire nation has been screened by chest x-ray examination. Every state in the union now has mass screening programs in operation; voluntary health organizations in every part of the land have been preaching the value of periodic chest x-ray examinations in every known way; and patients in hospitals and physicians' offices are being routinely screened for pulmonary disease. Most important, the people themselves appreciate the value and the importance of prevention in tuberculosis. Conceivably, the record of fourteen million chest x-rays taken in 1949, magnificent as it now appears, may pale on comparison with the record of a year from now or five years from now. Vast expansion can and will be achieved. The diagnostic responsibility, however, will be the physician's, and there is no doubt that he will find it a difficult one to discharge fully. His will be the all important task of directing the follow-up which will determine the etiology and significance of the lesions found in the x-ray films.

CONCLUDING REMARKS

One-half century ago the prevalence and pattern of tuberculosis in this country were such that even meager control efforts were bound to prove rewarding. Today, however, the pattern of tuberculosis is distinctly different: Evidence of the disease is more diffuse and more obscure, and we are beginning more and more to be confronted with the problem of the residual case. The progress we shall make in the future control of tuberculosis will therefore depend to a great extent on how well we can translate our knowledge of the changing pattern of the disease into indicated action. It will depend, in other words, on the intensity and breadth of our vigilance against the disease and on the vigor and completeness of our efforts.

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Seminars on Renal Physiology

Tubular Transport Mechanisms*

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FEW would now challenge the view that a fairly large number of more or less independent processes participate in the formation of urine by the glomerular kidney. Glomerular filtration is generally accepted to be a purely physical process by which a protein-free filtrate of plasma is delivered into the tubular lumina. As the filtrate passes through the tubules, certain substances are returned to the peritubular fluid by reabsorption while others are extracted from the interstitial fluid and delivered into the lumina by tubular excretion. The present discussion is primarily concerned with the active cellular processes which govern the movement of various solutes from the tubular urine to peritubular fluid and in the reverse direction.

ACTIVE TRANSPORT

A characteristic and apparently indispensable property of every living cell is its ability to establish striking differences in concentrations across the cell membrane. The unequal distribution of sodium and potassium ions between intracellular and extracellular water is a classical example. It is now clearly recognized that the effective separation of these two ions reflects more than a simple, selective permeability of the membrane.[1] The accumulation of potassium and the exclusion of sodium by most mammalian cells appears to be largely dependent upon active transport mechanisms which proceed at the expense of metabolically derived energy. Lacking any very specific knowledge of the metabolic processes involved, we encounter difficulties in attempting to define exactly what is meant by the term "active transport." One useful definition has been provided in a recent review on ion transport by Ussing[1] who states, " . . . we shall only speak of active transport when work has to be done to transfer ions across the membrane, whether this work is used to overcome a potential difference, a concentration difference or a combination of both."

The establishment of a concentration gradient does not always represent a result of active transport. This is exemplified by the following experiments of Kempton and Chambers.[2] It may be recalled that the frog kidney receives blood from two more or less independent sources, the glomeruli being supplied by the aorta and the proximal tubular segments by the renal portal vein. When the glomeruli are perfused via the aorta with Ringer's solution containing ammonium chloride, the pH of the glomerular filtrate passing through the tubules can be decreased from its usual value of 7.4 to 6.3. If the renal portal vein is perfused simultaneously with Ringer's solution containing the basic dye, neutral red, the dye accumulates in the proximal tubular lumina in a concentration some forty times that of the perfusing solution. Very similar results are obtained with the sulfonic acid dye, phenol red (phenolsulphonphthalein). On the other hand, when the tubular urine is made alkaline by perfusing the glomeruli with Ringer's-bicarbonate, the accumulation of neutral red ceases while that of phenol red continues essentially unchanged. In short, neutral red appears to move only toward a more acid solution whereas the movement of phenol red occurs independ-

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ently of the hydrogen ion concentration in the tubular urine. These findings suggest that we are dealing with active transport by the tubule only in the case of phenol red. Convincing evidence to support this assumption was provided by similar studies performed in kidneys poisoned with cyanide. Neutral red accumulated in the acid tubular urine of a poisoned kidney as readily as before, but all transport of phenol red ceased. Obviously, the movement of neutral red depended entirely upon the presence of a hydrogen ion gradient across the tubule while that of phenol red was intimately dependent upon the metabolic activity of the tubule cells.*

Intermediate storage of the transported material within the renal tubule cells does not appear to be a feature of most active transport systems. When phenol red transport is observed directly either in tissue cultured explants of chick mesonephros[3] or in isolated renal tubules of certain cold-blooded animals,[4] striking concentration gradients are established across the tubular epithelium without any apparent staining of the cell contents. In contrast, the basic dyes such as neutral red readily gain entrance into the cells, combine with cellular structures and, consequently, behave as vital stains. The tubular epithelium may actually represent a very real barrier to the free diffusion of materials even in the direction that they are normally transferred by active processes. The interesting studies of Shannon on the toadfish illustrate this point.[5] Over a wide range of plasma concentrations the maximal rate of phenol red excretion by the tubules was found to be the same whether the concentration of free dye in the plasma was higher or lower than that in the tubular urine. From these observations it may be concluded that all of the dye crossing the tubular epithelium is transported by an active process of limited capacity and that the dye cannot diffuse across the cells even in the direction of a favorable concentration gradient.

* The objection may be raised that the cells contributed to the transfer of neutral red by exchanging H^+ for NH_4^+ in order to establish the necessary hydrogen ion gradient. However, had the experiment been performed differently, i.e., by perfusing the glomeruli with Ringer's solution already adjusted to pH 6.3, the same results would almost certainly have been obtained.

TUBULAR REABSORPTION

Of the hundreds of compounds examined thus far by clearance technics only a few such as inulin, thiosulfate and ferrocyanide appear to be excreted solely by glomerular filtration. The majority are cleared from the plasma at rates either lower or higher than the filtration rate. The quantity of any particular material filtered at the glomeruli can be readily calculated as the product of the filtration rate and the concentration of freely diffusible material in the plasma.[6] When the quantity recovered in the urine is less than the filtered load, it may be presumed that the remainder has been reabsorbed by the tubules; recovery of more than the filtered quantity indicates tubular excretion, providing synthesis of the material has not occurred in the kidney. It should be appreciated that carefully performed clearance studies permit very precise estimates of such tubular activities.

Glucose is representative of the normal filterable constituents of plasma which must be conserved by tubular reabsorption. Micropuncture studies on amphibian[7] and mammalian[8] kidneys have shown that the concentration of glucose in the glomerular filtrate is identical with that in the plasma water. However, the concentration declines rapidly toward zero as the filtrate passes through the proximal tubular segment and only trace amounts of glucose appear in the normal urine. In clearance studies the quantity of glucose presented to the tubules for reabsorption can be increased by progressive elevation of the plasma glucose. Under such a circumstance glucose reabsorption continues to be essentially complete until the maximal capacity for reabsorptive transport is reached.[9,10] When the filtered load exceeds the maximal rate of transfer the excess glucose is quantitatively recovered in the urine. Extensive studies in the dog[9,10] and man[6] have shown the maximal rate of

glucose reabsorption (Tm_G) to be remarkably constant for any individual on repeated examinations. In the well hydrated dog fairly large variations in the glomerular filtration rate fail to affect the constancy of Tm_G. This finding has been interpreted to indicate that essentially all of the glomeruli are functioning in the state of adequate hydration and that variations in the filtration rate reflect altered filtering activity of all the glomeruli rather than a change in the number of active nephrons. This must be contrasted with the frog kidney in which glomerular intermittence can be visualized directly.[11]

Both fructose and galactose, unlike glucose, are only partially reabsorbed from the glomerular filtrate In the dog not more than 70 per cent of the filtered fructose is reabsorbed even at very low plasma levels.[12] Gradual elevation of the plasma level to over 300 mg. per cent is accompanied by increased reabsorption but an increasing fraction of the filtered load escapes into the urine as saturation of the transport mechanism is approached However, no true reabsorptive maximum for fructose has yet been demonstrated. Similar results have been obtained with galactose in the rabbit, cat, dog and man.[13] Attempts to demonstrate mutual interference for reabsorption between glucose, fructose and galactose have not been impressive but the reabsorption of all three can be partially or completely blocked by the administration of phlorhizin. Sucrose and xylose, two sugars which are metabolically inert when administered intravenously, are reabsorbed only to the extent of 20 to 25 per cent at low plasma levels[14] and this limited reabsorption can be completely blocked by adequate doses of phlorhizin.[15] It is of interest, however, that sufficient elevation of the plasma glucose to produce glucosuria seriously interferes with xylose reabsorption.[16]

Tubular reabsorption of the non-essential amino acids, glycine, alanine and glutamic acid, has been examined in the dog by Pitts.[17] Glycine is fairly completely reabsorbed at low plasma levels but its rate of excretion increases with rising plasma levels and an apparent maximal rate of transfer is approached only when the load presented to the tubules exceeds the quantity reabsorbed by a considerable margin. Glycine transport was found to be independent of the urine pH within the range 6.3 to 7.2 and was not influenced by the administration of phlorhizin. Rather similar results were obtained with alanine and glutamate[18] except that the plasma levels of these amino acids were never raised sufficiently high to approach saturation of the transfer mechanism. Beyer and his associates have used specific microbiologic assay methods in studying the excretion of a number of essential and non-essential amino acids in the dog.[19–22] Well defined maximal rates of transport were demonstrated for two of the basic amino acids, arginine and lysine. On the other hand, essentially complete reabsorption of tryptophane, leucine, isoleucine, valine, histidine, methionine, threonine and phenylalanine was observed even when plasma levels were raised to ten to fifty times their normal values. Competition for reabsorption was demonstrated for the following pairs of amino acids: lysine and arginine, histidine and arginine, leucine and isoleucine. Glycine infusions had no effect on the reabsorption of either leucine, isoleucine or arginine. These investigators conclude that at least three separate reabsorptive mechanisms operate in the transfer of amino acids, one handling the basic amino acids, arginine, lysine and histidine; the second, certain monoamino-monocarboxylic acids such as leucine and isoleucine; and the third, glycine.[22] It should be emphasized that such mutual interference may be an important factor conditioning the efficient utilization of intravenously administered mixtures of amino acids and protein hydrolysates.

The clearance of creatine in the dog and man is a curvilinear function of the plasma level.[23] Essentially no creatine appears in the urine at normal plasma levels but its clearance increases to values slightly below the rate of glomerular filtration when the

plasma level is raised to 20–40 mg. per cent. In normal adult males the elevation of serum creatine also depresses the reabsorption of its nitrogenous precursor, guanidoacetic acid.[24] Glycine, alanine and probably glutamic acid appear to compete with creatine for reabsorption in the dog.[18] Since such competition is demonstrable even at the levels of endogenous creatine, Pitts[17] has rightly stressed the possible hazards of interpreting metabolic studies on creatine production in experiments involving the excessive administration of amino acids.

The normal clearance of urate in man has been repeatedly shown to be about 10 per cent of the simultaneous filtration rate. Recently, Berliner and his associates[25] have succeeded in demonstrating a well defined and stable reabsorptive maximum for uric acid. Since the urate Tm in man is in the order of 15 to 20 mg./min./1.73 sq. M body surface area, the tubular reabsorptive mechanism is almost certainly never saturated in the normal individual. Consequently, it is of interest that a fraction of the filtered urate always escapes reabsorption. The rate of endogenous urate excretion is significantly increased by the administration of glucose, salicylates and phenol red[26] and by diodrast.[27]

Definite and reproducible reabsorptive Tm values have been demonstrated for both ascorbic acid[28,29] and inorganic phosphate[30,31] in the dog and man. The renal excretion of both compounds resembles that of uric acid in that a significant fraction of the filtered material escapes reabsorption long before saturation of the reabsorptive mechanism is reached. Since saturation is probably never achieved in the normal individual, it is unlikely that transport capacity is the prime determinant in the regulation of plasma levels of either ascorbic acid or phosphate. The reabsorption of ascorbic acid can be completely blocked temporarily by simultaneous loading with either *p*-aminohippurate or glucose.[32] Pitts and Alexander[30] have also reported that the phosphate Tm can be reduced 40 per cent by saturation of glucose reabsorption. The administration of phlorhizin in the latter situation abolishes the inhibitory effect of glucose and phosphate reabsorption may then actually increase to values somewhat higher than in the control periods.

Sulfate reabsorption in the dog[33] appears to present a unique situation. Essentially all of the filtered sulfate is reabsorbed at a normal plasma level and filtration rate. A slight increase in the filtered load rapidly exceeds the reabsorptive capacity and the excess sulfate appears in the urine. Further elevation of the plasma sulfate to ten to twenty times its normal value does not result in any additional reabsorption. Since the Tm is approximated even under normal conditions, it is quite conceivable that in the case of inorganic sulfate the reabsorptive capacity is the important factor in maintaining the normal plasma concentration within a fairly limited range.

TUBULAR EXCRETION

The conservation of valuable filterable constituents of plasma by tubular reabsorption is obviously an essential feature of the glomerular kidney. With a few noteworthy exceptions, glomerular filtration and incomplete tubular reabsorption account for the elimination of the various recognized metabolic waste products. However, in the bird and reptile[34] in which uric acid is the principal end product of nitrogen metabolism and must be excreted with a minimum of water, the tubular excretion of uric acid is an active and well developed process. In aglomerular species, tubular excretion obviously represents the only possible pathway for renal elimination.

The physiologic role of tubular excretion in man remains obscure. While a limited capacity for the tubular excretion of exogenous creatinine has been clearly demonstrated,[35] the participation of this transport system in the excretion of endogenous creatinine is less certain, largely because of the relatively non-specific analytic methods commonly employed. When the total creatinine-like chromogens of plasma and urine are estimated by the Jaffé reaction, the

clearance of "endogenous creatinine" closely approximates the glomerular filtration rate in normal subjects[36] and consequently has been used as a convenient measure of this function in a number of clinical studies. However, rather different results have been obtained with the apparently specific enzymatic method for creatinine devised by Miller and Dubos.[37] Approximately 20 per cent of the Jaffé reactive material in normal plasma represents non-creatinine chromogens whereas essentially all of the urinary chromogen appears to be true creatinine. In a series of unpublished observations on normal and hypertensive subjects[38] the clearance of "true" endogenous creatinine was found to exceed the filtration rate by an average of 25 per cent, a value only slightly below that obtained with moderately low plasma levels of exogenous creatinine.[35] Thus, while it seems probable that true endogenous creatinine is excreted in part by the tubules, the quantity actively transported must amount to somewhat less than 0.3 gm. per day in the average adult human. Recent studies in the dog by Beyer et al.[39] have also demonstrated the limited tubular excretion of N′-methylnicotinamide, one of the principal metabolic products of nicotinic acid.

It is therefore somewhat surprising to find that a variety of foreign compounds are removed from the plasma by tubular excretion at rates much greater than those for any known endogenous substances. The first convincing evidence of tubular excretion was in fact provided by the studies of Marshall and his colleagues[40,41] who found that the quantity of phenol red excreted by the dog exceeded by far that which was delivered to the kidneys in a filterable state. The tubular excretion of phenol red has since been shown to occur in a variety of aglomerular and glomerular kidneys and a limiting rate of active transport (Tm) has been clearly established.[5,42] Among other compounds actively excreted by the tubules of man and dog are diodrast (3,5-diiodo-4-pyridone-N-acetic acid), *m*- and *p*-aminohippuric acid, *p*-acetylaminohippuric acid and hippuran (*o*-iodohippuric acid), all of which are cleared at essentially identical rates at low plasma levels in any individual.[43] Since the efficiency of the cellular mechanism responsible for their transport is such that approximately 90 per cent of the material delivered to the kidneys is excreted in the urine,[44,45] their rate of clearance at low plasma levels may be regarded as a measure of the effective renal plasma flow. The small fraction of material which escapes into the renal venous blood is probably derived largely from that portion of the total renal blood flow which perfuses non-excretory tissue. Saturation of the transport mechanism occurs at high plasma levels and the maximal rates of diodrast and *p*-aminohippurate (PAH) transport have been employed widely in recent years in evaluating the mass of functional renal tissue in normal[46,47] and pathologic states. Certain precautions which must be exercised in interpreting such data in the diseased kidney have been emphasized by Earle.[48]

The tubular excretion of penicillins F, G and X has been demonstrated in the rabbit and man[49] and accounts for the rapidly falling plasma levels of these antibiotics. Although simultaneous comparisons have not been made, the three penicillins appear to be cleared at about the same rates as diodrast or PAH. Penicillin K is excreted much more slowly.

The mechanisms responsible for transporting strong electrolytes have been considered in detail by Pitts[50] and Berliner[51] in preceding articles of this series. Of particular interest is the recent demonstration that the renal tubules are capable of both reabsorbing and excreting potassium.[52,53] This finding may seem to cast doubt on the interpretation of many clearance studies in which unidirectional transport has been assumed. While one cannot exclude the possibility that substances other than potassium are handled similarly by the kidney, convincing evidence is lacking and numerous observations oppose the idea that this is a common renal mechanism.

COMPETITION FOR TRANSPORT

Only a few representative reabsorptive and excretory processes have been presented in the preceding sections. The total number of compounds known to be actively transferred across the tubular epithelium is sufficiently great to make unlikely the assumption that a specific cellular mechanism exists for the transport of each. Shannon[54] has advanced the logical hypothesis that certain cellular elements of the transport systems, available in constant but limited quantities, determine the maximal rates of transfer. Therefore, the extent to which compounds of similar chemical or physicochemical properties share a single transport mechanism of limited capacity should be revealed by the demonstration of typical competitive phenomena. When mutual interference can be demonstrated between two or more compounds of similar chemical structure actively transported in the same direction, true competition may be tentatively inferred.* Such is probably the case in the reabsorption of the amino acid pairs, arginine-lysine and leucine-isoleucine,[22] or in the tubular excretion of PAH and the other hippuric acid derivatives.[43] Less certain examples of true competition are represented by glucose and ascorbic acid,[32] glucose and xylose[16] and creatine and glycine.[17] Apparent competition for transport in the same direction, but involving compounds unrelated by structure, has been reported for glucose and inorganic phosphate,[30] glucose and uric acid[55] and between diodrast and each of the following: phenol red,[46] the hippuric acid derivatives,[43] exogenous creatinine[56] and penicillin.[57] Undoubtedly the most puzzling examples of apparent interference are those involving what would seem to be unrelated compounds normally transported in opposite directions, for example, diodrast and uric acid[27] or PAH and ascorbic acid.[32] It is difficult at present to conceive of a likely cellular element which would be shared in the reabsorptive transport of uric acid and the tubular excretion of diodrast, unless it is assumed that the energy available for cellular transport represents an important limiting factor.

The fact that certain results obtained by clearance methods appear to be more confusing than helpful may stem from the manner in which such phenomena are customarily examined in renal studies. The differentiation of true competition from non-competitive interference requires that both the absolute and relative concentrations of the two potential competitors be examined over fairly wide ranges, a procedure which rarely is practicable in an uninterrupted clearance study. Consequently, the demonstration under a limited set of conditions of interference by one compound with the transport of another does not constitute very rigorous proof of competitive inhibition in the usually restricted sense.

The action of carinamide® (4′-carboxyphenylmethanesulfonanilide) may be cited as a possible example of another type of transport inhibition. Originally developed as an effective agent for delaying the renal excretion of penicillin,[58] carinamide® has since been found to depress the tubular excretion of phenol red and PAH as well.[59] No effect was observed on the several reabsorptive processes examined. Studies on the accumulation of phenol red by kidney slices of the guinea pig[60] indicate that carinamide® acts as a true competitive inhibitor. Therefore, it may be concluded that carinamide® competes with phenol red, PAH and penicillin for combination with a cellular element of the tubular excretory mechanism. Unfortunately, clearance studies on carinamide® are inconclusive. Some evidence has been presented to indicate that it is excreted by glomerular filtration alone[59] while other observations suggest the possibility of

* "True competition" is used here in the same sense as in studies on enzyme inhibition. In competitive inhibition the inhibitor is regarded as competing with the substrate for specific groups of the enzyme and, consequently, the apparent decrease in activity of the enzyme depends on the relative concentrations of both the inhibitor and substrate. In non-competitive inhibition the inhibitor inactivates the enzyme by combination with groups not directly concerned with the substrate; hence, inactivation depends only on the inhibitor concentration.

tubular excretion[61,62] or reabsorption.[62] Perhaps the most attractive explanation at the present time is that originally proposed by Beyer,[63] namely, that carinamide® is capable of combining with an essential cellular element of the excretory mechanism but is refractory to excretion, thereby producing a so-called "renal blockade."

HORMONAL CONTROL OF TRANSPORT

In normal dogs prolonged administration of testosterone,[64] thyroxine[65] or anterior pituitary extracts[66] results in considerable enhancement of the tubular transport capacity for diodrast. Removal of the glandular hypophysis causes a permanent reduction in glomerular filtration, renal blood flow and the tubular excretion of diodrast or PAH.[67,68] However, in this situation neither thyroid, gonadal or adrenal cortical hormones are capable of overcoming completely the effects of hypophysectomy. The administration of growth hormone for nine to twelve days does restore completely the depressed renal functions of hypophysectomized dogs and can elevate those of normal animals to twice the control values.[69] In such studies the question necessarily arises as to whether the hormonal effects reflect an alteration of the transport kinetics or of the morphology of the functioning nephrons. It has now been definitely established that a number of steroid compounds stimulate the growth of the renal tubules;[70] prolonged administration of testosterone propionate to mice increases the kidney weight roughly in proportion to body size, but histologic studies reveal a disproportionate hypertrophy of the proximal convoluted tubules.[71] Since similar morphologic changes may well account for the chronic effects of other renotrophic factors, caution should be exercised in interpreting such results as relevant to the normal humoral control of renal function, at least insofar as finer regulatory adjustments are concerned. Acute increases in the Tm_{PAH} have been reported in man following administration of large doses of cortisone, while large doses of adrenocorticotrophic hormone apparently decrease the tubular reabsorption of endogenous urate and inorganic phosphate.[72]

Epinephrine has no effect on the glucose Tm in man[6] and large doses of insulin cause only an occasional slight lowering of the reabsorptive capacity in the normal dog[10] or man.[73] The glucose Tm in diabetic patients has been found to be normal, or slightly elevated relative to the glomerular filtration rate, and was reduced by insulin in all ten of the patients examined by Farber, Conan and Earle.[73] Lambert and his associates[74] have recently reported the interesting observation that intravenous injections of the water-soluble glucoside of desoxycorticosterone can reduce the glucose Tm of man by as much as 30 to 40 per cent. However, since desoxycorticosterone acetate[75] does not possess this property, the authors suggest that the observed effect may be due to the glucoside structure rather than a true hormonal action.

The phosphaturia and hypophosphatemia induced by parathyroid extract may logically be considered to result from the suppression of phosphate reabsorption. Unfortunately, renal studies performed at normal or moderately elevated plasma levels of inorganic phosphate have been inconclusive for technical reasons. A number of unpublished observations have failed to reveal any significant effect of parathyroid hormone on the maximal rate of phosphate reabsorption.

METABOLIC ASPECTS OF TRANSPORT

Since active transport has been defined in part as a process involving the expenditure of metabolic energy, some consideration must be given to the underlying biochemical mechanisms. It is hardly surprising, in the light of our present knowledge of intermediary metabolism, to find that relatively little progress has been made in this field of investigation. By way of review it may be said that we possess a fairly detailed knowledge of the important energy-yielding reactions of anaerobic and aerobic glycolysis, some insight into the nature of

certain coupled reactions through which energy is conserved, and a limited number of model biosynthetic reactions in which the energy source is well defined. The manner in which metabolic energy is translated into a complex physiologic function such as osmotic work remains almost totally obscure and consequently the description of active transport in terms of specific biochemical reactions gives at best only a fragmentary picture.

Available biochemical information does permit the construction of tentative hypotheses to explain the reabsorption of glucose (cf.[76] for review). Since glucose must undergo phosphorylation before entering the metabolic pathways leading to glycogen synthesis or degradative breakdown, it is reasonable to assume that this is the initial event in glucose transport. The conversion of glucose to glucose-6-phosphate within the luminal margin of the cell may be regarded as a device for establishing a favorable concentration gradient for glucose across the cell membrane. The cell membrane is apparently impermeable to the phosphorylated intermediate which, therefore, does not escape back into the lumen. The accumulated hexose phosphate must in turn be hydrolyzed by an appropriate phosphatase and the resulting glucose discharged through the peritubular margin of the cell. The orderly transport of glucose would obviously require a definite spatial orientation of the enzymes governing the two processes. The glucose phosphorylating enzyme, presumably a hexokinase, is abundantly present in the kidney although its distribution within the cell is unknown. However, the demonstration by histochemical methods of an intense localization of phosphatase near the luminal border of the proximal convoluted tubule[77] is in accord with the proposed scheme. The inhibition of glucose phosphorylation by phlorhizin in respiring kidney extracts has been accepted by many as additional supporting evidence (cf.[78] for review). In any event, it should be recognized that any scheme proposed at the present time represents little more than a working hypothesis for the planning of future studies.

The mechanisms responsible for transporting compounds other than glucose pose even more perplexing problems since they involve the interaction of compounds of uncertain chemical potentialities with undefined components of the cell. The important role of ion exchange mechanisms in the acidification of urine and in the tubular excretion of potassium has been reviewed recently by Pitts[50] and Berliner.[51] Binkley[79] has recently suggested that a similar mechanism may underlie the cellular transport of a much greater variety of compounds. He has isolated from the kidney an abundant lipoprotein complex possessing glutaminase activity and having many of the properties of certain ion exchange resins of the carboxylic acid type. Although no evidence has yet been presented to establish its relationship to renal function, the possibility suggested is an intriguing one.

It is reasonable to assume that transport consists of a series of two or more reactions, the last of which yields as a final product the parent compound. At least one of these reactions must involve the participation of an energy donating system. Certain generalizations concerning the source of energy utilized in active transport appear to be warranted. Richards and Barnwell[80] first noted that cyanide could abolish promptly and completely the accumulation of phenol red in the tubular lumina of excised frog kidneys. This striking dependence on aerobic oxidative reactions has since been demonstrated for the transport of phenol red in cystic explants of the chick mesonephros,[81] isolated tubules of the flounder[82] and thin slices of guinea pig kidney[60] and for *p*-aminohippurate transport in rabbit kidney slices.[83] Active transport can be sustained in such kidney preparations only with a continuous and abundant oxygen supply and ceases when cell respiration is interrupted by inhibition of the cytochrome-cytochrome oxidase system. Little evidence supports the view that anaerobic glycolysis makes any appreciable contribution to the energy

requirements of active transport. Beck and Chambers[84] have observed that iodoacetate retards the accumulation of phenol red by chick mesonephros and that such inhibition can be partially prevented by the prior addition of D-lactate or pyruvate. Since these metabolites are products of anaerobic glycolysis which arise subsequent to the reaction presumably blocked by iodoacetate, viz., the dehydrogenation of glyceraldehyde phosphate, the authors concluded that anaerobic glycolysis plays an important role in phenol red transport. However, a true reversal of iodoacetate inhibition cannot be obtained and it is probable that the effects of iodoacetate are attributable to the inhibition of certain cytochrome-linked oxidative enzymes of the citric acid cycle rather than a selective action on the triose phosphate dehydrogenase.[82]

It has become increasingly evident in recent years that special energy-rich phosphate compounds serve as the mediators of energy between various biologic systems.[78,85] The formation of adenylpyrophosphates during the oxidative reactions of glycolysis provides a device for channeling metabolic energy into biosynthetic processes and such physiologic functions as muscular contraction, neural transmission, etc. Consequently, it is reasonable to assume that cellular transport is empowered in a similar fashion. Recent observations on the effects of 2,4-dinitrophenol (DNP) appear to support this view. Dinitrophenol, at concentrations as low as 5×10^{-5} M, effectively blocks the generation of energy-rich phosphates without depressing the respiration of kidney enzyme systems.[86] The transport of phenol red by isolated fish tubules[87] and the accumulation of PAH by rabbit kidney slices[83] are reversibly inhibited by the same concentration of DNP. Closely related nitrophenols which are inactive as inhibitors of aerobic phosphorylation fail to interfere with cellular transport. Clearance studies in the dog show that a single intravenous injection of DNP (10 mg./kg. body weight) reversibly depresses the tubular excretion of phenol red, diodrast or PAH without significantly altering the rate of glomerular filtration, renal plasma flow or urine volume.[88] Shapiro[89] has previously reported the inhibitory action of phlorhizin on several dehydrogenases of the citric acid cycle and on aerobic phosphorylation in kidney, as measured by the synthesis of creatine phosphate, and has suggested that interference with essential phosphorylation reactions underlies the inhibitory action of phlorhizin on both tubular reabsorptive and excretory processes. Although the utilization of phosphate bond energy seems clearly implicated in tubular excretion, the situation is generally less certain with respect to tubular reabsorption. In the dog the reabsorption of glucose, glycine,[88] inorganic phosphate, sodium or potassium[90] was unaffected by the usual intravenous dose of DNP. The failure of DNP to inhibit these tubular reabsorptive processes cannot be adequately explained but it has been suggested that the transport systems operating in opposite directions may differ significantly either in their energy requirements or in the manner in which phosphate bond energy enters into the reactions of transport.[88]

It is highly unlikely that compounds such as phenol red, diodrast or PAH undergo direct phosphorylation during transport. Phosphate bond energy probably participates in their excretion through the activation of one or more of the cellular components of the transport system. Recent studies on PAH excretion may throw some light on the nature of the cellular elements which limit certain transfer processes to a maximal rate. Thin slices of rabbit kidney cortex, when incubated in the Warburg apparatus in a saline medium containing dilute PAH, accumulate this compound against a considerable concentration gradient.[83] Such accumulation by tissue slices is confined to the kidney cortex and occurs only in an aerobic environment. When various metabolic intermediates were added to the suspending medium, some degree of respiratory stimulation generally occurred but the rate of PAH accumulation increased significantly (approximately doubled) only

with the addition of acetate or certain of its immediate precursors. Other oxidizable substrates either failed to alter or actually depressed PAH transport. These findings *in vitro* suggested the possibility that acetate, or some product derived therefrom, represents one of the rate-limiting cellular components of the PAH excretory mechanism. Confirmatory evidence was provided by clearance studies in the dog.[91] The maximal rate of PAH transport was increased 42 to 85 per cent above the control values by adequate intravenous infusions of sodium acetate (66 micromoles/kg./min. or more). Such an acute increase in the transport capacity occurred independently of changes in the filtration rate or urine flow; nor was it related to minor changes in the plasma electrolyte pattern since similar changes induced by bicarbonate infusions failed to elicit more than a minimal response. Of particular interest was the finding that the glucose Tm was completely unaffected by such acetate infusions.

Little can be said concerning the exact role of acetate in PAH transport or the extent to which acetate participates in other transport systems. However, it is fairly certain that acetate as such is not the functional cellular component. Analysis of various biosynthetic reactions involving this intermediate have disclosed that acetate must first be raised to a more reactive state through interaction with energy-rich phosphates.[92–95] Consequently, it may be tentatively assumed that so-called "active acetate" is the common product of energy and acetate metabolism which plays some important role in PAH transport. The foregoing observations may also serve to point out at least two mechanisms by which phosphate bond energy may be utilized in cellular transport, either by direct phosphorylation of the transported compound, as in the case of glucose, or by activation of an essential cellular component, as in the case of PAH.

SUMMARY

Endogenous and exogenous compounds of a great variety and number are transferred across the tubular epithelium by active cellular processes. In the mammalian kidney the conservation of valuable filterable constituents of plasma is accomplished by tubular reabsorption while glomerular filtration and incomplete reabsorption account for the renal elimination of many of the known waste products of metabolism. Although various foreign compounds are known to be actively excreted by the tubules and have proven useful in the clinical evaluation of renal function, the physiologic role of tubular excretion in man remains obscure. Little is known about the normal hormonal regulation of tubular transport aside from those factors which have been found to modify the excretion of electrolytes and water.

Active transport has been defined as a process in which work must be done by the cells to transfer materials across the tubular epithelium against a chemical potential. The energy utilized in active cellular transport is apparently derived largely from aerobic metabolic reactions and is channeled through energy-rich phosphate bonds. Uncertainty surrounds the mechanisms by means of which phosphate bond energy is translated into osmotic work; it is suggested that such energy may be used for direct phosphorylation of the transported compound in some systems and for the activation of essential cellular elements in others.

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Case Reports

Myasthenia Gravis*

Review of the Literature and Report of a Case of Malignant Thymoma

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ALTHOUGH the symptoms of myasthenia gravis were described as early as 1685 by Willis,[89] the disease was not fully recognized until the end of the nineteenth century. Wilks,[88] Erb,[21] Oppenheim,[64] Goldflam,[28] Hoppe[38] and Jolly[49] firmly established the entity on the basis of its symptoms, signs and absence of bulbar lesions at autopsy. A recently observed case which was carefully studied both clinically and at necropsy is here reported.

CASE REPORT†

J. S., a sixty-five year old white male, was admitted to the New Britain General Hospital as a patient on August 26, 1947, in a state of profound weakness. The remote past history was negative. One and a-half years prior to admission he noted a non-productive cough, vague substernal distress and malaise. He had several similar bouts for five months. On June 18, 1946, he had an x-ray taken of his chest. This revealed a prominent mediastinal shadow on the left which was interpreted as a lymphoblastoma, probably Hodgkin's type. The following month over a period of eleven days the patient was given 2,090 roentgen units through an anterior mediastinal port and the same amount through a posterior mediastinal port. During the early treatment he complained of increased substernal discomfort and some difficulty in swallowing and breathing. Subsequently he felt somewhat improved. Because of the complexity of his symptoms, hospitalization for evaluation was advised.

He was admitted to the Grace Hospital in New Haven, Connecticut, on August 4, 1946, and discharged on August 20, 1946, improved. The physical examination was negative. An x-ray of the chest showed no increase in hilar markings (indicating a decrease in size of the tumor mass following the previous roentgen therapy). The only positive findings were a positive blood Wassermann test which was considered to be a biologically false positive result, and an intravenous pyelogram interpreted as showing "downward displacement of the left kidney caused by a retroperitoneal mass." He was given 5,000,000 units of penicillin and a course of x-ray therapy. The latter was given through four ports in the lumbar region for a total of 5,460 roentgen units. Two months later therapy was repeated to the same region through four ports with a total of 3,024 roentgen units. He was readmitted on February 10, 1947, and discharged on February 24, 1947, improved. He stated that he had been fairly well since discharge until he developed an influenza-like illness with hoarseness and malaise. An x-ray of the chest showed no change from the previous admission. Intravenous pyelogram revealed "marked reduction in the amount of displacement of the left kidney." He was treated with oral liver and iron and discharged symptom-free. His third admission occurred on March 20, 1947, and he was discharged on April 11, 1947, improved. On admission he complained of increasing weakness, dysarthria and dysphagia. He received 1,680 roentgen units to the cervical posterior spine and an equal amount to the upper dorsal spine with apparent temporary improvement. The fourth admission was on May 16, 1947, and he was discharged on June 11, 1947. He had developed progression of his previous symptoms together with mental depression. At home he had been receiving neostigmine with marked improvement. In the hospital he required large oral doses plus parenteral injections at two to four-hour intervals. Following discharge he received maintenance doses of neostigmine but there was gradual

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deterioration until admission to the New Britain General Hospital on August 26, 1947. For thirty-six hours prior to his admission he received 12 ampules of neostigmine without much improvement. On admission he appeared in extremis with marked muscular weakness and inability to speak above a whisper.

Laboratory data were as follows: Hemoglobin 87 per cent, white blood cell count 5,600, neutrophiles 77, segmented neutrophiles 9, lymphocytes 14; urinalysis negative; blood, per 100 milliliters, non-protein nitrogen 43 mg., serum protein 6.1 gm., blood cholesterol 178 mg. He was given neostigmine (1 mg.) with atropine sulfate ($\frac{1}{200}$ gr.) intramuscularly every three hours without improvement. He refused to have a Levine tube inserted. He was given 1,000 cc. of amigen. About twenty-four hours after admission he suddenly developed respiratory distress, became cyanotic and expired.

At autopsy the sternum was adherent to an irregular flattened tumor mass, measuring 8 cm. in diameter, which was also fixed to the anteromedial surfaces of the upper lobes of both lungs and to the intermediate underlying mediastinal structures. The tumor was firm and was surrounded by dense, gray, fibrous adhesions. On section the tumor extended into the lung parenchyma and into the adjoining tracheobronchial lymph nodes. The body of the tumor was pinkish gray. There were two slightly flattened nodules adherent to the leaf of the diaphragm on the right. A third nodule was in the mid-portion of a dense adhesion on the right diaphragm. On section these nodules resembled the primary tumor. The adrenal cortex was a mottled yellow-brown and was somewhat thinner than normal. Examination of the brain was not performed and sections of muscle were not taken.

Sections of the primary tumor and one lymph node showed the tumor to be separated by partially hyalinized fibrous trabeculae into larger and smaller irregular cell nests made up of large numbers of polyhedral cells with light pink staining, somewhat hyperchromatic nuclei. Within the tumor nests were typical small lymphocytes. The histologic picture resembled that of the thymus. In some areas there were tiny, partially hyalinized, somewhat acellular cell nests simulating Hassall's bodies. Lymphorrhages were absent.

Anatomic diagnosis: Malignant thymoma of anterosuperior mediastinum involving adjacent lung and tracheobronchial lymph nodes; tumor implantation upon leaf of right diaphragm with adhesions to lung; brown atrophy of heart; brown pigmentation, atrophy and slight irregular fatty degeneration of liver; moderate congestion of all organs; slight lipoid depletion of adrenals.

Symptoms and Signs. Myasthenia gravis occurs most commonly from the second to the fourth decade. In 125 cases reviewed by Harvey[36] the youngest patient was three and the oldest seventy-five. There were 57 per cent females and 43 per cent males. The onset was before the age of forty in 87 per cent of the females and in 56 per cent of the males. Acute upper respiratory infections, emotional tension and hyperthyroidism may precede or be associated with the disease.

The onset of symptoms is generally gradual but they may develop suddenly and cause vascular abnormalities to be suspected. The disease may have its first manifestation as weakness of a generalized nature or localized to the arms or legs. Sensory disturbances such as pain in the eye, numbness and tingling of various areas may precede or accompany the first manifestations of weakness.[36] Viets[83] reviewed 175 cases of myasthenia gravis at the Massachusetts General Hospital: ptosis and diplopia occurred in 43.4 per cent; next in frequency, at 34.2 per cent, was general weakness due to involvement of the arms and legs; dysphagia and dysarthria occurred in 20.1 per cent; weakness of the muscles of the neck was found in 2.3 per cent. The nasolabial folds are erased, the eyelids droop, the forehead is smooth at times and on other occasions is wrinkled into exaggerated contractions in an effort to correct the ptosis of the eyelids. The lips are parted and when the patient tries to smile the upper lip lifts, exposing the teeth.[29] The muscular weakness becomes more pronounced following exercise; it is relieved by rest.

Of eighty-seven cases reviewed by Kennedy and Moersch[41] (prior to neostigmine) twenty-seven patients had remissions; the average duration of the remission was 2.2

years. In three instances the remission lasted five, seven and fifteen years, respectively. In Harvey's[36] series it was noted that only occasionally does the disease regress once neostigmine therapy has been introduced.

The effect of pregnancy on myasthenia gravis is usually favorable. Viets, Schwab and Bazier[80] observed that most patients experience a definite remission in symptoms; relapses, if they occur, are mild. Relapses usually occur in the first trimester. Therapeutic abortion has not been found necessary nor is it advised, provided the patient can be maintained under prostigmine control.

Kowallis, Haines and Pemberton[45] noted the somewhat similar symptoms of myasthenia gravis and exophthalmic goiter and point out the specificity of response to prostigmine in patients with myasthenia gravis. Thorn and Eder[75] reported on two cases of myasthenia gravis associated with Graves' disease; they collected six cases from the literature. In their two cases the myasthenia improved simultaneously with improvement in the thyrotoxicosis. McEachern and Parnell[52] reported on eight cases including two of their own. In the latter two cases the myasthenia became worse while the hyperthyroidism improved.

Examination of the heart has shown no abnormalities in a group of fourteen patients with myasthenia gravis.[73] The carotid sinus was active in every case. The reaction to the administration of $\frac{1}{50}$ gr. of atropine was also normal.

Female patients give a history of exacerbations of muscular weakness and fatigability during the menstrual period; symptoms are most pronounced during the first day or two of the period.[55]

Etiology. While myasthenia gravis has been noted following a variety of illnesses and in association with endocrine disorders, no specific etiology has yet been found. Rothbart[67] mentions a family in which four brothers had myasthenia gravis. Nevertheless, the familial incidence in this disease is low.

Embryology. The epithelial elements of the human thymus are derived from two distinct embryologic sources; these are the third endodermal branchial pouch (exclusive of the portion which constitutes the primordium of the parathyroid) and the ectodermal cervical sinus. The cervical sinus is the primordium of the primitive thymic cortex and the source of Hassall's corpuscles. The endodermal thymus gives rise to the syncytial cytoreticulum of the gland. The thymic lymphocytes are of mesenchymal origin and they secondarily invade the gland after the entrance of vessels and connective tissue cells.[62]

Physiology. The neurophysiologists have contributed much to an understanding of the disorder. The resemblance of curare poisoning stimulated Walker[84,85] to use, with great effect, physostigmine and prostigmine in the treatment of myasthenia gravis. Forty years previously Jolly,[49] on theoretic grounds, had suggested the use of physostigmine. He demonstrated that subjecting the affected muscles to a tetanizing faradic current produced progressively weaker muscular contractions. After a short period of rest the muscle returned to its former state of excitability. Pritchard[66] in stimulating the ulnar nerve of a human myasthenic subject compared the lessening muscular response to that of Wedensky inhibition.

In 1937 Lanari[46] and Fraser, MacGeorge and Murphy[25] independently demonstrated the peripheral action of prostigmine in myasthenic patients. They injected prostigmine into an artery which was occluded above the site of injection; only the tested extremity recovered its strength while the rest of the musculature remained myasthenic until venous circulation from its site was opened. Harvey and Lilienthal[35] confirmed these observations.

Feldberg and Vartiainen[23] demonstrated that eserine strongly sensitizes ganglion cells to injection of acetylcholine. Stronger concentrations produce an effect resembling the paralytic effect of nicotine. Dale, Feldberg and Vogt[14] showed that acetylcholine

was released at voluntary motor nerve endings. Measurements have shown that there is no increase of cholinesterase in the blood of myasthenics, and following the injection of prostigmine there is a reduction of cholinesterase.[31]

Harvey and Lilienthal[35] suggest that the circulation of a curare-like inhibitor may be the primary fault. Torda and Wolff[76] also postulate a curare-like agent in myasthenia gravis. Barondes[3a] attempts to explain the curare-like disturbance in myasthenia on the basis of an abnormality in lipid metabolism.

Dale[15] summarizes the existing possibilities underlying the disorder of myasthenia gravis: (1) It is not yet excluded that cholinesterase may be abnormally abundant in effective relation to the acetylcholine depots, although failure to find it in excess where it can be measured has rendered this unlikely. (2) There may be a defect of synthesis of acetylcholine and in consequence a defective replenishment of the depots at nerve endings. (3) The sensitiveness of the muscle end plates of acetylcholine may be lowered as in partial poisoning by curarine; it may even be thus reduced by the action of an endogenous curarizing poison.

Several substances have been found to enhance the action of acetylcholine. Ephedrine sensitizes the muscular response to adrenaline and to the stimulation of adrenergic nerves.[26] Guanidine also enhances the muscular response to acetylcholine in the experimental animal;[24] it does not lower the blood cholinesterase.[58] Potassium has been shown to have an anticurare action.[90]

The possible role of the endocrine glands in myasthenia gravis has been suggested by animal experiments. Houssay[39] demonstrated that removal of the adrenals would produce hyperplasia of the thymus. Adler[2] reported experiments in which transplantation of juvenile thymus tissue or injection of thymus extracts in dogs produced a picture similar to myasthenia gravis of man, which was improved by prostigmine. Bomskov and Milzner[10] were not able to reproduce his results. McEachern[51] was unable to demonstrate any secretory function of the thymus.

Pathology. Weigert[86] in 1901 reported the association of a tumor of the thymus with myasthenia gravis. Bell,[5] Norris[61] and Lievre[47] reviewed the reported autopsied cases of myasthenia gravis and observed an incidence of approximately 50 per cent with thymic enlargement or tumor. In 17,000 autopsies at Bellevue, Symmers[72] gathered twenty-five thymic tumors or enlargements; two of the tumor cases clinically resembled myasthenia gravis. Homburger[37] noted forty-one instances of tumor or enlargement of the thymus among 6,000 autopsies at the New Haven Hospital; of these, two were associated with myasthenia gravis. Miller[56] encountered five cases of myasthenia gravis among 16,300 autopsies at the Johns Hopkins Hospital.

Sloan[71] has pointed out the great variations in the total weights of normal thymus glands and the variations in actual amounts of thymic tissue and adipose tissue present in glands of similar weight. He noted changes resembling those of myasthenia gravis in the thymus of a few patients with acromegaly, Addison's disease and hyperthyroidism.

The term thymoma, introduced by Grandhomme,[32] has been compared by Ewing[22] to the term lymphoma. Those tumors which metastasize cannot be distinguished on a histologic basis from those which do not. The tumors are composed of two cell types: one is identical with small lymphocytes seen in lymph nodes; the other is oval, three to four times as large as a lymphocyte, contains faint acidophilic cytoplasm and a nucleus which stains lightly with hematoxylin.[60]

Meggendorfer's[53] report in 1908 of a malignant thymic tumor associated with myasthenia gravis was questioned by Bell[5] because of a lack of detailed description. However, in recent years there has been an increasing number of reports regarding malignant thymoma in patients with myasthenia gravis.[16,57,60,71] In the majority of cases the metastases are local and within

the chest cage. Hardymon and Bradshaw[32a] reviewed 132 cases of myasthenia gravis and found four cases of malignant thymoma. Crosby[13a] summarized 166 cases of malignant tumors of the thymus and reported only two associated with myasthenia gravis. Since the origin of the cells of the thymus is still in doubt there is difficulty in classification of the thymic tumors and in evaluating the type of tumors associated with myasthenia gravis. Although Keynes[43] believes that the epithelial thymic tumor is the only one associated with myasthenia gravis, others[65,72] have cited cases of different groups including perithelioma, lymphosarcoma and epithelioma.

Buzzard[12] described clumps of lymphocytes in skeletal muscle, heart, liver, adrenals and thyroid tissue; he termed these lymphorrhages. They had previously been described and called muscle metastases.[40,86] Lymphorrhages have been found in two-thirds of the autopsied cases of myasthenia gravis.[61] Several observers have noted involvement of the heart by lymphorrhages; these are sometimes associated with edema within and between the muscle bundles.[4,11,12,68]

There have been no consistent changes in the central nervous system. Early reports mentioned chromatolysis of cells of the cranial nerves,[87] and others mention diminution of basophil substance throughout the central nervous system.[59] Most articles record negative findings.

Diagnosis. The characteristic history and physical examination may be confirmed by several test procedures. The prostigmine test in myasthenia gravis was elaborated by Viets, Schwab and Mitchell[70,77,78] and confirmed by others.[27,34] The former group determines the most noticeable sign, such as ptosis, then injects intramuscularly 1.5 mg. of prostigmine methylsulfate to which has been added $\frac{1}{100}$ gr. atropine sulfate, and grades the objective and subjective response at ten-minute intervals for one hour. The response usually appears after fifteen to twenty minutes. Tether[74] prefers the intravenous injection of neostigmine (0.5 mg.) without atropine, given within a timed minute. Improvement occurs almost immediately and is maximal within five minutes. In occasional doubtful cases 1 mg. is given on the following day. Atropine sulfate, usually 0.6 mg., should always be ready for subcutaneous or intravenous injection whenever side effects become manifest. Goodman[30] observed severe parasympathetic-like reactions following the ingestion of 45 mg. of prostigmine. The only fatality following neostigmine is recorded by Merrill;[54] it came after the injection of 0.5 mg. neostigmine methylsulfate for a diagnostic test. Death was considered by Tether[74] as probably resulting from a drug idiosyncrasy. This case emphasizes the need for immediate use of atropine when side effects develop. The drug is very valuable in diagnosis but it must be used with due caution. Viets and Schwab[79,83] have utilized fluoroscopic examination in patients with dysphagia, the barium sulfate being observed both before and after the injection of prostigmine. Kennedy and Wolf[42] pointed out the antagonistic action of quinine to prostigmine in myasthenia gravis. Harvey and Whitehill[33] utilized quinine to increase the symptoms of weakness. Eaton[17] proposes 10 gr. of quinine sulfate in two or three doses three hours apart; definite muscular weakening is usually observed one hour after the second dose.

Bennett and Cash[6] introduced curare sensitivity as a test for myasthenia gravis: $\frac{1}{5}$ or $\frac{1}{20}$ of the average adult intravenous dose of curare (5 to 15 mg.) causes a profound exaggeration of symptoms in myasthenia patients. The peak reaction occurs within two minutes of the injection. The test is terminated within two to three minutes by intravenous injection of 1.5 mg. prostigmine methylsulfate with $\frac{1}{100}$ gr. of atropine sulfate. Both the quinine and curare test are used only in cases having questionable or absent muscular weakness. They might be extremely dangerous in a patient already weakened from his disease. Eaton[18] has cautioned against the use of curare in convulsive shock treatment of patients with psychiatric disorders who

have myasthenia gravis. It is conceivable that in the weakened patient intravenous curare might cause death. Electrical stimulation of muscle or nerve may be used as a diagnostic clinical test but this method has been chiefly of value in investigating the disease. The finding of lymphorrhages in muscle biopsies has been a confirmatory test for myasthenia gravis.

X-ray and fluoroscopy of the chest is valuable in detecting tumors of the thymus. In a series of 191 consecutive cases studied at the Mayo Clinic the roentgenologists, using lateral and oblique positions, made a definite diagnosis in thirty cases, an incidence of 15.7 per cent.[13]

Adams, Power and Boothby[1] found no consistent abnormality in metabolism in myasthenia gravis. Creatinuria above normal was observed in seven of thirty cases.

Differential Diagnosis. Keschner and Strauss[44] have ably summarized the differential diagnosis. The early phases of the disease may simulate neurasthenia. The character of the weakness, relief with rest and the remissions favor the diagnosis of myasthenia gravis. Polyneuritis has objective sensory changes, muscular atrophies, reflex and electrical changes. The muscular dystrophies and atrophies have typical changes in musculature and are progressive; the associated muscular weakness is not so rapidly relieved by rest. Patients with degenerative lesions of the bulb have paralyses, atrophies, fibrillations (particularly of the tongue), reactions of degeneration in muscles involved and a rapidly progressive course. Those with pseudobulbar paralyses have evidence of upper motor neuron lesions, repeated attacks of apoplexy and often a history suggestive of arteriosclerosis.

The initial diagnosis is frequently encephalitis. An adequate history, physical examination and spinal fluid examination differentiates the two. Syphilis involving the bulb is excluded by the negative reaction of the blood and spinal fluid to the Wassermann test and by the normal gold curve.[11]

The asthenia of hyperthyroidism and Addison's disease are usually characteristic; the use of a diagnostic test with prostigmine is of value. The association of hyperthyroidism with myasthenia gravis must be borne in mind.

TREATMENT

Drug Therapy. At present neostigmine is the most specific drug. Eaton[19] has reviewed the establishment of the optimum dosage of the drugs used in myasthenia gravis. The basic requirements with neostigmine bromide (15 mg. tablets) are determined by giving the drug every two to three hours during the day and adjusting the dosage. Sixty-four per cent of the cases required three to nine tablets. Once the basic dose is estimated a trial is given with the addition of ephedrine $\frac{3}{8}$ gr. two or three times daily; it is ordinarily not used after 4:00 P.M. because of insomnia. Harriet Edgeworth,[20] herself a myasthenic, first noted the value of ephedrine. Glycine is seldom used now. Supplements of either guanidine hydrochloride, eight to ten of the 125 mg. tablets, or potassium chloride 25 to 35 gm. daily have been used. However, few of Eaton's patients have continued with these drugs. Viets[82] noted that nearly all patients with guanidine have paresthesias about the mouth and fingertips and have given up its use. Potassium chloride is disagreeable in taste and has a mild effect on the myasthenia in a small percentage of patients. Di-isopropyl-fluorophosphate is less effective than neostigmine.[12a] The reactions of neostigmine are gastrointestinal cramps, nausea, diarrhea, syncope, increased salivation and perspiration. These are controlled by atropine $\frac{1}{100}$ gr. or tincture of belladonna 10 to 20 drops fifteen minutes before the premeal dose of neostigmine.[19] Patients usually have more distress on an empty stomach.

Recently tetra-ethylpyrophosphate was introduced by Burgen and co-workers[11a] for the treatment of myasthenia gravis; this drug is a powerful anticholinesterase which has a longer and more even action than

neostigmine. The maintenance dose in three patients has varied between 8 to 12 mg. daily given in two or three doses orally.

The more severe cases may have choking with aspiration pneumonia and even respiratory paralyses requiring the use of a Drinker respirator.[19] Frequent injections of neostigmine are often needed. Viets[81] has used up to 31 mg. intramuscularly at hourly doses in the course of twenty-four hours. In patients having difficulty in swallowing, Eaton[19] advises the use of feeding by stomach tube of a liquid mixture avoiding excess carbohydrates (because of their weakening effect as personally observed by Harriet Edgeworth).

Moehlig[50] obtained relief in a patient seriously ill with myasthenia gravis from injections of desoxycorticosterone acetate and also from implantation of pellets of this drug. Further reports of similar cases are required for adequate evaluation of this therapy.

X-ray Therapy. As was noted in the case reported, x-ray therapy at the beginning not infrequently aggravates the symptoms. Eaton[19] reserves judgment as to the value of roentgen irradiation of the thymus. If the patient is too ill to withstand surgical treatment, he often advises roentgen therapy. Viets[82] cites experience with fifteen cases none of which had any beneficial effect.

Surgery. The early attempts to alter the course of myasthenia gravis by surgery were performed by Sauerbruch.[3,63,69] The first cure of a case of myasthenia by removal of a thymic tumor was reported in 1939 by Blalock, Mason, Morgan and Riven.[7] Since that time the number of reports on surgery of the thymus gland has increased steadily; the larger series have been reported by Blalock and co-workers,[8,9,36] Clagett and Eaton[13] and Keynes.[43] Surgery has been facilitated by better pre- and postoperative management with neostigmine, oxygen, tube feeding, penicillin and the use of a Drinker respirator. Long and Allen[48] reviewed 129 reported cases of myasthenia gravis which had been operated upon: seventeen essentially well; twenty-four considerably improved; twenty-seven moderately improved; twenty-one slightly improved; others were unimproved, unclassified or died. Keynes[43] believed the prognosis was poor for those with tumor but in Clagett's[13] experience the patients with tumor have done far better than those with hyperplasia, as far as the myasthenia is concerned. Clagett and Eaton[13] summarized 103 reported cases operated upon: twenty-five tumors were found; of the 103 cases thirteen were "operative" deaths and one "postoperative" death. In Harvey's[36] review of thirty-two cases, seven had circumscribed tumors and twenty-five hyperplastic thymus tissue; three died in the immediate postoperative period.

Blalock[9] states that he knows of no way to predict preoperatively which patients will be helped by thymectomy. His results suggest that patients with benign thymic tumors are most apt to be benefited. They also suggest that patients who have had the disease for short periods of time are more apt to show dramatic improvement than are those who have had the disease for an extended period. Poer[65] reported a case of removal of a malignant thymic tumor with marked improvement of the myasthenia. The subject of thymectomy requires further extensive study before any final decision can be rendered as to its role in myasthenia gravis.

SUMMARY

Myasthenia gravis, a disorder without known etiology, has been studied extensively by the neurophysiologists; it is thought to be associated with either a defect of synthesis of acetylcholine or with a lowered sensitiveness of the muscle endplates to acetylcholine, possibly because of a curare-like substance. Hyperplasia or benign and malignant tumors of the thymus occurs not infrequently. Microscopically lymphorrhages in skeletal muscle or in various organs are frequently found. The disorder occurs at any age, in frequent association with upper respiratory infec-

tions, and occasionally with hyperthyroidism. It is characterized by gradual, at times sudden onset with weakness usually involving the eyelid, muscles of mastication, larynx, and less frequently the extremities. It is aggravated by exercise, quinine and curare. It is relieved by rest, neostigmine and often by pregnancy. There are normally remissions and exacerbations. The disease has been confused with neurasthenia, polyneuritis, muscular dystrophies, muscular atrophies, degenerative lesions of the bulb, pseudobulbar paralyses, epidemic encephalitis, syphilis involving the bulb, hyperthyroidism and Addison's disease. Careful evaluation of the history, examination, response to quinine, curare, and neostigmine, and spinal fluid examination usually suffice to make the diagnosis. The chief drug therapy is neostigmine which is sometimes combined with ephedrine. Recently tetra-ethylpyrophosphate, which has a longer and more even action than neostigmine, was introduced in the treatment of myasthenia gravis. X-ray therapy has been used but thus far without great success. Surgical removal of the thymus whether it is composed of hyperplastic tissue or tumor in a significant number of cases gives definite improvement. Further study is necessary for proper evaluation of surgery of the thymus gland in myasthenia gravis. The thymus gland may show involvement by hyperplasia and benign or malignant thymoma in a significant number of patients with this symptom-complex; it is not, however, the etiologic agent of this disease.

The case presented emphasizes the early symptoms and signs with x-ray evidence of a mediastinal mass, muscular weakness, increasing symptoms at the beginning of x-ray therapy followed by temporary improvement, exacerbation of symptoms with an upper respiratory infection, temporary relief by neostigmine and finally a respiratory type of death. At autopsy the patient was found to have a malignant thymoma with local extension and metastases of the tumor limited to the thoracic cage.

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Meningitis Due to Pasteurella Other Than Pasteurella Tularensis and Pasteurella Pestis*

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MICROORGANISMS of the genus pasteurella rarely cause meningeal infection in man. We are reporting two cases of meningitis due to pasteurella, other than Past. tularensis or Past. pestis, which we have observed between March, 1943, and May, 1948. We also wish to review some of the relevant literature and to consider chemotherapy and treatment with antibiotic agents.

The genus pasteurella includes three species other than Past. tularensis or Past. pestis, namely, Past. pseudotuberculosis, Past. hemolytica and Past. multocida.

Not much concerning infection with Past. pseudotuberculosis and Past. hemolytica need be written here. Of the fourteen known instances of infection of man with Past. pseudotuberculosis, none has been a case of meningitis.[1] As far as our knowledge goes, also, no case of infection of man with Past. hemolytica has been reported.

The type species Past. multocida includes a group of microorganisms, the hosts of which are numerous animals and fowls, including the cat, dog, cow, horse, sheep, pig, rabbit, rat, chicken, duck, reindeer, buffalo and panther. Bacteria of this group cause hemorrhagic septicemia, a disease of common occurrence among birds and mammals.[2,3] The incidence of infections with Past. multocida in man, on the other hand, is low. Since 1938, the year of publication of Regamey's important report, the number of authenticated cases of pasteurellosis of man has risen to nearly thirty.[4,5] Ten of these (Table 1), including our two which are reported herein, have been cases of meningitis.[5–13]

CASE REPORTS

CASE 1. A white male bartender, thirty-two years of age, entered the hospital on March 8, 1943, because of convulsive seizures. He had had epileptic convulsions every month since the age of ten years. For the preceding nine years, however, he had been relatively free of seizures and had been accepted for military duty. The attacks had recurred, and the patient had been discharged from the army four months prior to his admission to the hospital. Seizures usually had been preceded by dizziness and followed by nausea, vomiting and severe, bifrontal headache. Three months before the patient's entry to the hospital, his right upper eyelid had become

TABLE 1
SUMMARY OF REPORTED CASES OF PASTEURELLA MULTOCIDA MENINGITIS

Author	Number of Cases	Year	Associated Pathology	Outcome
Claudius[6]	1	1925	Skull fracture	Death
Constandache and Franke[7]	1	1929	Skull fracture	Recovery
Vincent[8]	1	1930	Following use of	Death
Levy-Bruhl[9]	..	1934	infected rabbit muscle as a cerebral hemostatic	
Regamey[5]	1	1938	Skull fracture	Recovery
Le Chuiton and others[10]	1	1939	Skull fracture	Recovery
Kapel[11]	1	1942	Postoperative meningocele	Death
Fólmer and Have[12]	1	1943	Postoperative; brain surgery	Recovery
Tomić-Karović and Ivanović[13]	1	1944	None apparent	Death
Zeller and others	2	1949	None apparent	Recoveries

* Work done in George Washington University Medical Division of Gallinger Municipal Hospital, Washington, D. C.

lacerated in the course of one of his seizures and ultimately plastic repair had been required.

On examination the patient responded only sluggishly to interrogation. Temperature, pulse and respirations were normal. The blood pressure was 125 mm. of mercury systolic and 80 diastolic. Other than a few minor contusions and lacerations about the head and neck, nothing further of significance was found.

Two epileptic seizures occurred in the course of the four days after the patient's admission to the hospital. On the fourth day his temperature rose to 102°F. Nuchal rigidity and a positive Kernig's sign developed.

The cerebrospinal fluid was examined, therefore, with the following results: Leukocytes numbered 2,900 per cu. mm., 95 per cent of which were polymorphonuclear cells. The concentrations of sugar, protein and chloride were respectively 20.8, 225 and 644 mg. per 100 cc. The reaction of the cerebrospinal fluid was negative for syphilis and the colloidal gold curve was represented by the numerals 0000000000. Direct smear of the sediment revealed gram-negative pleomorphic bacilli. Culture of the original specimen was positive for a microorganism that later was identified at the National Institute of Health as pasteurella other than Past. tularensis or Past. pestis.

Examination of the blood and urine, and roentgenologic examination were not very enlightening. The concentration of hemoglobin was 11.5 gm. per cent per 100 cc. of blood. Erythrocytes numbered 3,900,000 and leukocytes, 10,200 per cu. mm. In the Arneth count there was a slight shift to the left. The Kahn reaction performed on the blood gave a negative result, as did each of several blood cultures. Examination of the urine disclosed nothing abnormal. Roentgenologic examination of the thorax gave no evidence of pulmonary pathologic change and a roentgenogram of the skull did not indicate the presence of fracture.

For the meningitis, 6 gm. of sulfadiazine was given by mouth immediately and 1 gm. was given every four hours for twenty-three days thereafter. The concentration of sulfadiazine in the blood averaged 10 mg. per 100 cc. throughout the treatment. Occasionally, crystals of sulfadiazine appeared in the urine. The urine, however, was kept alkaline by oral administration of 0.66 gm. of sodium bicarbonate every four hours. Gross hematuria or renal colic did not develop and treatment was continued.

The remainder of the patient's stay in the hospital was uneventful. The temperature remained elevated for four days but was normal thereafter. Signs of meningeal irritation disappeared concomitantly with the fever. The leukocyte count and protein content of the cerebrospinal fluid were still slightly elevated on the eleventh day following onset of the meningitis (33 lymphocytes per cu. mm. and 100 mg. per 100 cc., respectively) but were normal in the specimen of fluid obtained on the twenty-second day. Repeatedly, results of culture of the cerebrospinal fluid were negative. The patient had no further convulsive seizures and was discharged markedly improved on the thirty-fourth day following onset of the meningitis.

Case II. A white man, fifty-nine years of age, employed as a cook in a hotel, was referred to the hospital by his physician for diagnostic study. Two weeks before his admission to the hospital, the patient had returned home from work complaining of fever and general malaise. He had consulted a physician who had prescribed sulfadiazine, 1 gm. every four hours orally for several days. For a short while thereafter the patient had felt better. One week before his admission to the hospital another physician was reported to have diagnosed prostate gland trouble. Symptoms of fever and headache had persisted.

The patient again had consulted the original physician who then had noticed nuchal rigidity. The cerebrospinal fluid had been grossly cloudy. Leukocytes per cubic millimeter of this fluid had numbered 4,500, of which 97 per cent were polymorphonuclear cells. Microorganisms had not been seen on direct smear. Further bacteriologic studies had not been made. Significant findings elicited by a consultant in neurology were bilaterally absent superficial and deep tendon reflexes. The consultant had expressed the opinion that the patient was suffering from "a bizarre form of meningitis due probably to a yeast or fungus." Then penicillin, 50,000 units, had been administered intramuscularly every three hours and 1 gm. of streptomycin had been given per day, also intramuscularly, in divided doses.

On the patient's admission to the hospital he did not appear acutely ill, although he seemed drowsy and somewhat dazed. He was well oriented when aroused and answered questions intelligently. His temperature was 104°F., pulse rate 100 and the respiratory rate 20 per minute.

Neurologic examination disclosed slight weakness of the right facial muscles, bilaterally absent superficial and deep tendon reflexes, nuchal rigidity and a bilaterally positive Kernig's sign. Petechiae were not seen on the skin and mucous membrane. The only remaining physical sign of importance was diffuse enlargement of the prostate gland.

The most important set of laboratory procedures was examination of the cerebrospinal fluid. The fluid was cloudy and contained 5,200 leukocytes per cu. mm., of which 78 per cent were polymorphonuclear cells. The dextrose content was less than 10 mg. per 100 cc. and the concentration of protein was 481 mg. per 100 cc. Two plates of chocolate agar media were inoculated and pasteurella grew on both. Later, the microorganism was identified at the National Institute of Health as pasteurella other than Past. tularensis or Past. pestis.

Blood and urine were examined and roentgenologic examination of the thorax was made. The concentration of hemoglobin was 12 gm. per cent per 100 cc. of blood; erythrocytes numbered 4,000,000 and leukocytes 16,000 per cu. mm. On Arneth count there was a shift to the left. Serologic tests for syphilis gave negative reactions. No growth was obtained on initial blood culture. Albumin in the urine was graded 2 plus. An occasional granular cast per low power microscopic field and fifteen to twenty white blood cells per high power field were seen. Roentgenographic study, mentioned earlier, revealed no evidence of cardiopulmonary disease.

Management was that for inadequately treated purulent meningitis of unknown etiology. Since the identity of the microorganisms was unknown on the first day of hospitalization, on empiric grounds, 2 gm. of sulfadiazine were administered by mouth immediately and 1 gm. every four hours thereafter; also 1,000,000 units of penicillin was given intramuscularly every two hours. The amount of penicillin was reduced to 1,000,000 units every three hours on the sixth day of hospitalization and administration was discontinued on the ninth day. Sulfadiazine was administered until the fourteenth day.

The course was satisfactory. At no time during the patient's stay in the hospital did he appear acutely ill. The weakness of his right facial muscles disappeared by the second day of hospitalization, although signs of meningeal irritation persisted for five days. His temperature ranged between 100° and 102°F. for the first three days in the hospital. On the fourth day the patient was afebrile and remained so thereafter. As in Case I, pleocytosis of the cerebrospinal fluid and elevation of the concentration of protein persisted. On the thirty-eighth day of hospitalization, 54 lymphocytes were found per cu. mm. of fluid and the concentration of protein was 128 mg. per 100 cc. The colloidal gold curve was represented as follows: 3322211100. In spite of these abnormalities the patient was dismissed forty-five days after he had entered the hospital, subjectively and clinically much improved.

COMMENT

Identification of the Species of the Causative Microorganism. We do not know certainly that the causative microorganism in our cases was Past. multocida. The isolated strains died before pathogenicity for animals and studies for agglutination had been completed. However, the biochemical reactions of the microorganisms in question are compared in Table II with those of the standard species, Past. multocida, Past. hemolytica and Past. pseudotuberculosis. The reactions of our microorganisms corresponded fairly closely with those of the standard species but, of course, such reactions are not conclusive. (Table II.)

Certain reasons, nevertheless, justify the belief that the bacteria which we isolated were Past. multocida. In the first place, one investigator of much experience in identification of pasteurella microorganisms believes that Past. hemolytica is not distinct from Past. multocida.[1] Second, neither of our microorganisms was hemolytic. Third, since review of the literature indicates that Past. hemolytica is not pathogenic for man and since cases of meningitis due to Past. pseudotuberculosis have not been reported, it is unlikely that the causative microorganism in our cases was either of these.

Because Past. pseudotuberculosis is more resistant to penicillin than is Past. multocida, it has been suggested that determination of sensitivity, or of lack of sensitivity, to penicillin be used as a test to distinguish the microorganisms one from the other.[14] One

of our microorganisms was found to be extremely sensitive to penicillin (less than 0.039 unit of penicillin per cubic centimeter of culture medium was lethal). It is reasonable, therefore, to assume that this particular microorganism was Past. multocida.

TABLE II
REACTIONS OF ORGANISMS OF OUR CASES COMPARED WITH THOSE OF STANDARD SPECIES OF PASTEURELLA

Biochemical Reagent	Case I	Case II	Standard Species*		
			Past. multocida	Past. hemolytica	Past. pseudo-tuberculosis
Adonitol	....	0	0	.....	.....
Amygdalin	....	0	0	.....	.....
Arabinose	....	0	0	0	A
Dextrin	....	Tr	0	A	.....
Dextrose	A	A	A	A	A
Dulcitol	....	0	0	0	.....
Erythritol	....	0	0	.....	.....
Galactose	....	A	A	A	.....
Glycerol	....	Tr	0	±	A
Inositol	....	0	.....	A	.....
Inulin	0	0	0	0	.....
Lactose	0	A	0	A	.....
Levulose	A	A	A	A	.....
Maltose	A	0	0	A	A
Mannitol	A	A	A	A	A
Mannose	....	A	A	0	.....
Raffinose	0	Tr	0	A	.....
Rhaminose	....	0	0	0	A
Salicin	0	0	0	0	A
Sorbitol	0	A	A	A	.....
Starch	A	0	.....	.....	.....
Sucrose	A	A	A	A	±
Trehalose	....	0	±	.....	.....
Xylose	Tr	A	±	A	A
Citrate	....	0	.....	.....	.....
Lead acetate	0	0	+	.....	+
Litmus milk	Alk	0	No ch/sl A	No ch/sl A	Alk
Indole	0	+	+	0	0
Methyl red	....	0	.....	.....	+
Voges Proskauer	....	0	.....	.....	0
Gelatin	0	0	0	.....	No liquefaction
Nitrates	+	..	+	.....	+

* According to Bergey's Manual of Determinative Bacteriology.
Key: A = acid; 0 = no change; Tr = trace of acid; Blank = not tried; ± = some strains cause acidity, others do not; Alk = alkaline; No ch/sl A = no change or slight acid.

If after following the aforementioned line of reasoning, we can correctly assume that our microorganisms were Past. multocida, then our cases of meningitis, to the best of our knowledge, are the ninth and tenth to be reported in the world literature and the first two in the literature in English.

Pathogenesis. It is difficult to understand the pathogenesis of the less common forms of pasteurella meningitis when so few cases are available for study. In seven of the ten cases of Past. multocida meningitis, however, a history of either accidental or surgical trauma to the skull was elicited. (Table I.) In the cases of Constandache and Franke[7] and of Regamey,[5] a month or more elapsed between the time of cranial injury and the onset of meningitis. No adequate explanation for this long incubation period was given, but Regamey expressed the belief that the low pathogenicity of the causative microorganism for man was accountable. He thought the pathway of infection was from a latent focus in the pharynx or nasopharynx to the meninges by way of a direct communication at the site of fracture.

The route by which the infection traveled to the meninges in our cases is even more difficult to ascertain. The head injury received following the epileptic convulsion might have been of some etiologic importance in our first case. In the second case a history of head injury was not obtained and no focus of infection was demonstrated except possibly a focus in the prostate gland. The environmental and occupational histories were carefully investigated but proved unenlightening. We do not know the source of infection or portal of entry in either of our cases.

Prognosis. The prognosis for patients with Past. multocida meningitis is good. In our cases the disease was mild and the response to treatment was favorable. The fatality rate for all reported cases is 40 per cent. (Table I.) Concerning patients who died, however, it was difficult to decide whether the meningitis only or the underlying or associated intracranial pathologic condition was the more important cause of death.

Chemotherapy and Antibiotic Therapy. Evaluation of the effect which sulfadiazine or penicillin had on recovery of our patients is difficult for the following two reasons: (1) The patients might have recovered without specific treatment since recoveries were reported before sulfonamides and antibiotics were available.[4,7,10] (2) Both sulfadiazine and massive doses of penicillin were

used in one of our cases. There is suggestive evidence, nevertheless, that these agents contributed to the recovery of the patients. First, subjective and objective improvement was noted in both cases following institution of treatment. Second, the causative microorganism in one of the cases was found to be sensitive to penicillin.

Penicillin seems to be the preparation of choice in treatment of Past. multocida infections of man. Schipper[14] isolated a strain of Past. multocida from the throat of a wild Norway rat and determined that the microorganism was sensitive to as little as 0.1 unit of penicillin per cubic centimeter of culture medium. This compares favorably with the sensitivity of our strain. Furthermore, in seven cases of bronchiectasis and one case of empyema attributable to Past. multocida, Olsen[15] used penicillin with good results. The concentration of penicillin *in vitro* which was lethal for the microorganisms isolated in Olsen's cases, ranged from 0.1 to 0.8 unit per cubic centimeter. Likewise, other investigators have reported Past. multocida to be sensitive to penicillin.[16–18] The suggestion that sensitivity to penicillin be used as a rapid test for differentiating Past. multocida from other gram-negative rods has been mentioned. Penicillin also has been used successfully for treatment of Past. multocida infections in ducks and cattle.[19,20]

The sulfonamides have been used experimentally to protect mice against lethal doses of Past. multocida injected intraperitoneally. Both sulfadiazine and sulfathiazole have been effective.[21]

Schipper[14] and Needham[22] both found strains of Past. multocida to be somewhat resistant to streptomycin; the lethal concentration of streptomycin ranged from 4.5 to 25 mg. per cubic centimeter of culture medium. The causative microorganism isolated in our second case, on the other hand, was destroyed by 0.75 mg. of streptomycin per cubic centimeter. Conceivably, this antibiotic might have been tried therapeutically in our case.

SUMMARY

Two new cases of meningitis attributable to microorganisms of the genus pasteurella other than Past. tularensis and Past. pestis are reported herein. Although complete bacteriologic identification of the species of the causative microorganism in each case was impossible, the probability is strong that it was Past. multocida. In one case sulfadiazine was administered; in the other, both sulfadiazine and massive doses of penicillin.

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Scleredema*

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SCLEREDEMA adultorum was originally described by Buschke[1] in 1900. Subsequently 107 cases have been reported.[2–5] The majority of these reports have appeared in the German literature. The first presentation of this distinct clinical entity in this country was the classic paper of Epstein in 1932.[6] Altogether only twelve reports[3–14] were found in the American literature and, with but four exceptions, all appeared in local or dermatologic journals.

As is true in the case of many diseases with prominent skin manifestations, for many years scleredema was considered a dermatologic disease. Recently Vallee has stressed the systemic manifestations of this malady including pleural, pericardial and joint effusions.[3] Since the disease usually follows an infection, is treated by physicians other than dermatologists and is commonly diagnosed erroneously as scleroderma, a crippling and at times fatal disease, it is fitting that attention to scleredema be focused in other than dermatologic literature.

CASE REPORT

G. C., a forty-one year old white Latin-American male, was admitted on April 27, 1949, about three weeks after the onset of pain in the ankles, knees and fingers. No preceding febrile illness was recalled. Seven days after onset, when the arthralgias involved the low back, shoulders and wrists, the patient was bedridden. Three days following this he became asymptomatic and remained symptom-free until three weeks after the onset of the initial joint symptoms when he noted that the skin over his neck was thickened and tight. Within a few days this process spread to his face, shoulders, chest, back and abdomen.

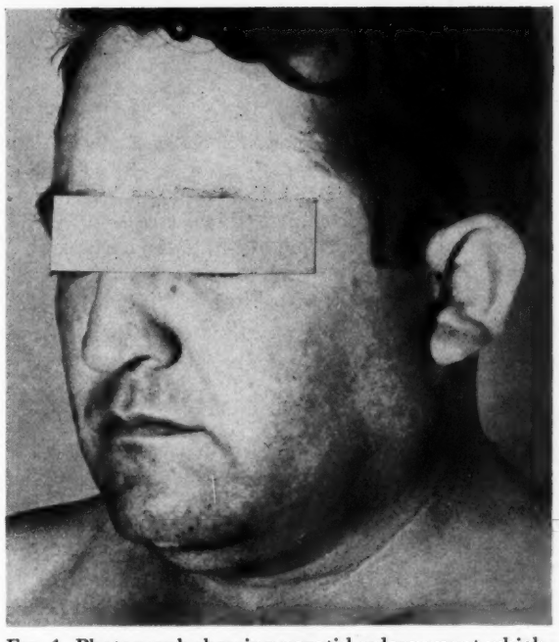


FIG. 1. Photograph showing parotid enlargement which was present bilaterally. Salivary gland involvement has never previously been recorded in scleredema.

There was no noticeable progression or regression of this edema after the initial spread and his general health was otherwise excellent. Bilateral parotid swelling was also observed simultaneously with the onset of the edema.

Physical examination revealed a well developed, rather obese male with a puffy "moon face" and striking bilateral parotid enlargement. (Fig. 1.) A hard, brawny, non-tender, non-pitting edema of the skin was noted in the areas mentioned. The hands and feet were free and the joints were normal. Aside from the skin involvement the physical examination was otherwise normal.

Examination of the blood and urine revealed no morphologic or chemical abnormalities. The sedimentation rate varied from 26 to 8 mm. per hour (Wintrobe). The Wassermann test

* From the Department of Medicine, Southwestern Medical School of the University of Texas, and the Department of Medicine of the Veterans Administration Hospital, Dallas, Tex. Published with permission of the Chief Medical Director, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the authors.

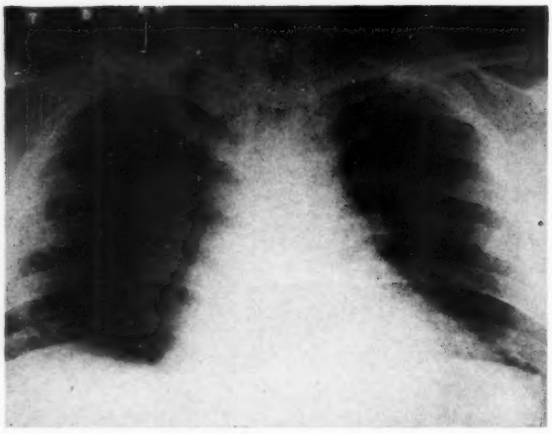


FIG. 2. Roentgenogram of the chest showing an unusual density outside the cardiac border that was more radiolucent than the remainder of the cardiac silhouette and extended from the diaphragm to the aortic arch.

was negative. Glucose-tolerance, liver and kidney function tests were all normal. Endocrinologic survey was negative. The basal metabolic rate, 17-ketosteroid excretion, sweat sodium[20] and epinephrine test for pituitary-adrenal cortical function[17,22] were normal.

Roentgenogram of the chest revealed a density outside the cardiac border that was more radiolucent than the remainder of the cardiac silhouette and extended from the diaphragm to the aortic arch. (Fig. 2.) The exact nature of this density was not known but it was interpreted as being an unusual fat pad. Skull x-rays revealed a normal sella turcica. Gastrointestinal series and barium enema were normal. Cardiac fluoroscopy revealed normal cardiac pulsations, no evidence of fluid or chamber enlargement and the density surrounding the heart which was again interpreted as an unusual collection and distribution of "fat" around the heart.

Skin biopsy on May 19, 1949, disclosed the classic findings of scleredema adultorum except that the perivascular infiltration consisted of polymorphonuclear leukocytes instead of lymphocytes, fibroblasts and plasma cells. Cresyl violet stain for the mucin-like substance in the cutis was negative. Repeat skin biopsy on July 26, 1949, revealed the identical findings except that the perivascular infiltration was now predominantly of the round cell type.

A trial of pyribenzamine therapy, 900 mg. per day in divided doses, was given from May 10, 1949, to June 1, 1949, without beneficial effect. Since the 11,17-oxysteroids have been shown to be efficacious in mesenchymal diseases,[15–17] an attempt was made to increase their endogenous production by the administration of epinephrine.[18] Accordingly, 350 μg. of U. S. P. epinephrine was given subcutaneously five times a day from June 2, 1949, to July 7, 1949. Shortly after the initiation of this therapy the patient noted increasing freedom from the constricting sensation secondary to the scleredematous process. Objectively, resolution of the edema, starting in the neck, was observed. Within three weeks the neck and face were free of edema and there was definite diminution in the edema over the chest, back and abdomen. The parotid swelling was unchanged. No further resolution was noted after three weeks although the therapy was continued for thirty-six days. Follow-up by mail in September, 1949, revealed no further involution of the edema after the initial improvement which occured during the first three weeks of the epinephrine therapy.

Scleredema (adultorum of Buschke) is a benign, diffuse collagen disease with predominant and ofttimes exclusive skin manifestations. After a variety of febrile illnesses, usually streptococcal infections but including influenza, mumps, scarlet fever, pneumonia, nephritis, rheumatic fever, pharyngitis and tonsillitis, a latent period of one to six weeks' duration follows. The dramatic skin manifestations usually appear abruptly but may be preceded by a short prodromal period characterized by myalgias, malaise and low grade fever. The first dermatologic sign is the appearance of a hard, brawny, painless, non-pitting edema commonly beginning at the nape of the neck and spreading by continuity to involve the face, scalp, shoulders, chest and back. Many times the process reaches the abdomen and buttocks. Although the extremities may be involved, it is characteristic that the hands and feet remain free of edema. The involvement is always symmetrical although one side may be more severely affected than the other. The maximum spread is usually reached within one to two weeks and is then followed by a static period or one of slow progressive involution. At the borders a sharp delineation between normal and involved areas is not found for the hard, lardaceous edema fades gradually into normal skin.

Dysarthria and dysphagia may result from involvement of the tongue and pharyngeal tissue. Parotid involvement has not been recorded previously but was present bilaterally in our patient. (Fig. 1.) The constricting sensation around the chest resulting from the enveloping edema may lead to mild dyspnea on exertion and restriction of movements of the affected parts. Although paresthesias at times have been mentioned, it is noteworthy that pain is never present.

The involvement of the face with consequent obliteration of bony landmarks and deeper folds gives the puffy "moon face" a characteristic mask-like expression. The edema may lend a pallor to the skin but the ivory hue of scleroderma is absent. The eyelids appear edematous and red when affected, and chemosis results from conjunctival involvement.

On palpation a surprisingly hard, brawny edema that does not pit on firm pressure is noted. The involved skin feels thickened, indurated and tightly bound down to the underlying tissue resulting in inability to pick the skin up in folds or move it freely over the subjacent tissue.

That the skin is not the only organ involved in scleredema was stressed by Vallee who pointed out the hydroarthrosis noted by other authors and reported cases with pleural and pericardial effusions. To one not cognizant of these manifestations of scleredema, this disease may present a difficult diagnostic problem.

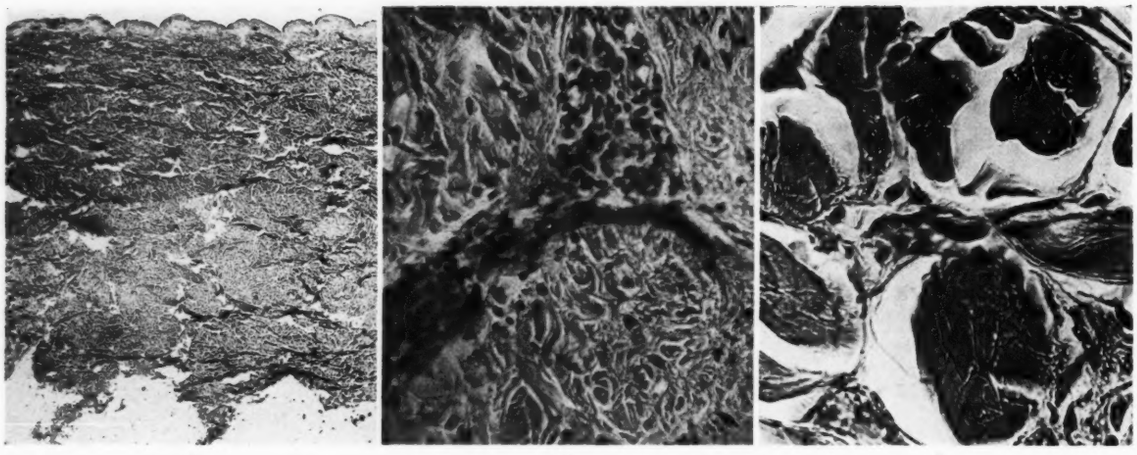
Although complete involution of the edema without sequelae usually occurs within eighteen months, the process may last from one month to several years. Rare cases have been observed in which isolated islands of edema have persisted as long as thirteen to twenty years. Characteristically, resolution follows a typical pattern. The last area affected is usually the first to resolve and the initial location of the edema is the last place to become free of edema. Under the influence of new noxious stimuli, relapses have occurred even after long free periods.

Laboratory examination has revealed no characteristic or diagnostic findings. The blood and urine have revealed no distinctive chemical or morphologic change. The 17-ketosteroid excretion has been normal. In our patient tests of adrenal cortical function including the sweat sodium,[20] 17-ketosteroid excretion and epinephrine test[17,22] were within normal limits.

Roentgen examination may reveal hydrothorax, hydropericardium and hydroarthrosis. Radiograph of the chest in our patient disclosed an unusual density surrounding the entire cardiac shadow. (Fig. 2.) This was not thought to be fluid and did not have the characteristics of a fat pad. Although not proven it is possible that the scleredematous process involved the pericardium. This finding did not disappear during a three-month period of observation.

Vallee surveyed the literature in 1946 and added his four cases to the ninety-nine previously reported, bringing the total to 103. Recent review disclosed four new cases[2,4,5] in addition to the one being reported, a total of 108 cases. Three additional cases were found in the foreign literature.[24–26] However, these journals were not available and therefore the cases are not included in this study. Although four of the five new cases were males, in all cases in which the sex is known there were thirty-seven males and sixty-five females. The disease has been recorded in infants and sexagenerians. However, the erroneous implication of the name scleredema adultorum is evident since 27 per cent of the cases occurred in the first decade and 50 per cent within the first two decades.

The histologic picture in scleredema shows several characteristic findings, the specific changes being located in the cutis and subcutaneous tissue. The derma is markedly thickened. (Fig. 3.) A slight perivascular infiltration of lymphocytes, fibroblasts and plasma cells is present in the superficial portion of the cutis, the deeper layers showing a more pronounced infiltration. (Fig. 4.) In the deeper portion of the cutis the collagen bundles are markedly hypertrophied, swollen, twisted and of



3 4 5

FIG. 3. Photomicrograph of the skin showing the marked thickening of the derma; fenestrated areas separating the collagen bundles are also seen.

FIG. 4. Photomicrograph showing the typical round cell perivascular infiltration consisting of lymphocytes, fibroblasts and plasma cells.

FIG. 5. Photomicrograph showing the markedly hypertrophied collagen bundles separated by clear spaces giving a fenestrated appearance.

unequal size. (Fig. 5.) They are separated from each other by a mucin-like substance the chemical nature of which is not known. This material does not stain with hematoxylin eosin but at times may appear brilliant red with cresyl violet dye. These clear spaces separating the enlarged collagen bundles result in a characteristic fenestrated appearance. This may also be found around blood vessels and the epithelial appendages. The process may extend to the subcutaneous tissue but the muscles appear normal. A derangement in the colloidal state of the collagenous system has been postulated by Klemperer et al.[19] to account for the disproportion between the histologic alteration and the impressive thickness and hardness of the skin. In support of this thesis they point out that the spontaneous recovery favors a transient colloidal imbalance within the collagenous tissues.

In the case being reported the first biopsy revealed the classic findings of scleredema save for the perivascular infiltration which was predominantly polymorphonuclear leukocytic. Dr. Jacob Furth, pathologist at this hospital, suggested that this biopsy may have been taken at an earlier phase of the disease than reported by others and suggested that biopsy at a later date would reveal the typical perivascular cells. Accordingly, two months later a repeat biopsy was done and the predicted changes were noted. This is the first time, to our knowledge, that a predominantly polymorphonuclear leukocytic perivascular infiltration has been demonstrated early in this disease.

The etiology of scleredema remains unknown. Further histochemical study on the mucin-like material in the cutis and subcutaneous tissue may clarify the pathogenesis of this disease. The general opinion is that the initial infection produces an agent responsible for the disease. This is supported by the knowledge that the disease most frequently follows a streptococcal infection. The possibility of derangement in the colloidal state of the collagen tissue has already been mentioned.[19,21]

Scleredema, with its excellent prognosis as to life and function, is most frequently confused with the edematous stage of diffuse scleroderma, a crippling and at times fatal disease.[6,14,21] This may account in part for the apparent low incidence of scleredema and the benignity of some of the reported cases of diffuse "scleroderma."

Although the edematous phase of scleroderma may closely mimic scleredema, there are many distinguishing features

between these two diseases. (Table 1.) Scleredema is preceded by an infectious disease and, after a latent period, begins characteristically on the neck and reaches maximum involvement by continuity within one to two weeks. Scleroderma has no constant relationship to preceding infection and frequently begins on the hands, feet and face, whereas the hands and feet are uninvolved in scleredema. Prodromal vasomotor symptoms are common in scleroderma and absent in scleredema. Spontaneous remission without atrophy or pigmentation is the rule in scleredema, whereas in scleroderma there is progressive involvement leading to hidebinding, atrophy, pigmentation and telangectasia.

Other conditions readily differentiated are trichinosis, myxedema, dermatomyositis, sclerema neonatorum, edema neonatorum and edema of obstructive, cardiac or renal origin.

TREATMENT

The evaluation of therapeutic agents in this disease, characterized by the universal tendency to spontaneous recovery, is hazardous. To date no therapy that produces unequivocal recovery has been disclosed in spite of the variety of agents tested including thyroid extract, anterior pituitary extract, typhoid-induced fever, removal of foci of infection, dihydrotachysterol, pyribenzamine, artificial heat and massage.

The recent demonstration of the therapeutic effectiveness of the 11,17-oxysteroids (Compound E) and adrenocorticotrophic hormone (ACTH) in rheumatoid arthritis[15] led to their trial in other collagen diseases including rheumatic fever and lupus erythematosis.[16,17] The results reported thus far have been encouraging.

Since Compound E and ACTH were not available, an attempt was made to increase the endogenous production of this adrenal cortical hormone by the administration of epinephrine. It has been shown in animals[18] and man[17] that the administration of epinephrine stimulates the anterior pituitary gland to increase the output of ACTH which in the presence of normal adrenals results in the outpouring of the 11,17-oxysteroids by the adrenal cortical cells. Accordingly, the patient was given 350 μg. of U. S. P. epinephrine subcutaneously five times a day for thirty-six days to achieve this effect.

TABLE 1
DIFFERENTIAL DIAGNOSIS

	Scleredema	Scleroderma (Edematous Phase)
Clinical		
Preceding infection	Almost always; usually streptococcal; followed by latent period 1–6 wk.	Rare; no constant relationship
Prodromal period	Infrequent; malaise, myalgia, low grade fever	Common; athralgia with pain, swelling and stiffness of joints; vasomotor symptoms in extremities in ⅓ of the cases
Edema	Rapidly progressive, maximum involvement within 2 wk.	Slow progressive involvement
	Hands and feet never involved	Hands and fingers (sclerodactylia) involved at onset or early in the disease
	Involved areas pale but not dead white	Dead white or ivory colored; sheen or waxy hue present
Sequelae	None; no pigmentation, atrophy, or telangectasia	Atrophy, pigmentation, telangectasia, hidebinding frequent
Course	Spontaneous recovery within months to years; usually within 18 mo.	Relentless, progressive atrophy and hidebinding common; spontaneous remission rare; 10% mortality; 75% protracted course
Pathology		
Epidermis	Uninvolved, no atrophy	Involved; increased pigmentation; atrophy
Cutis	Round cell perivascular infiltration collagen fibers markedly enlarged and separated by clear spaces giving a fenestrated appearance	Edema, homogenization fibrosis and sclerosis of collagen; proliferation of connective tissue with sclerosis and atrophy
Blood vessels	No endarteritis	Sclerosis and obliteration of blood vessels, some vessels thrombotic
Muscles	Normal	Edema and loss of cross striations early, degenerative changes later

It is interesting that shortly after the initiation of this therapy the patient noted subjective improvement. He no longer felt constricted by this taut, brawny edema. Objectively, there was a disappearance of the edema first on the neck and then on the face, and a diminution of the edema in the other involved areas. The usual pattern of spontaneous recovery is that the last area affected is the first to clear. The antithesis

occurred in this patient. Epinephrine was stopped at the end of one month since no further resolution occurred after three weeks of therapy. The homeostasis of the pituitary-adrenal system[18] may have prevented further effectiveness of the epinephrine. It is known that an elevated corticoid level in the blood tends to depress the anterior pituitary gland and decrease its output of ACTH. Intermittent therapy might have been more efficacious.

The objective loosening of the skin and disappearance or decrease in edema associated with the subjective feeling of increased freedom from constriction bear some resemblance to the alleviation of stiffness noted with the use of Compound E and ACTH in rheumatoid arthritis.[17] Thorn et al. have postulated a decrease in some "stiffness factor"[17] related possibly to the effect of the adrenal cortical hormones on tissue permeability.[23] Since epinephrine therapy has not proven as beneficial in the treatment of rheumatoid arthritis as Compound E or ACTH,[17] a more dramatic effect on scleredema may be anticipated with the use of these agents.

SUMMARY

Since 1900 when scleredema was described by Buschke a total of 107 cases have been reported with but few exceptions in the foreign literature. The disease is characterized by the appearance of brawny, nonpitting, painless edema usually starting on the neck and rapidly spreading by continuity to the head, chest and back. Although the abdomen and extremities may be involved, the hands and feet are spared. The disease is preceded by a febrile illness, usually a streptococcal infection, and commonly undergoes spontaneous involution without sequelae within three to eighteen months. Systemic involvement, including pleural, pericardial and joint effusions, has recently been stressed. Twenty-seven per cent of the cases occurred within the first decade and females were involved more frequently than males.

The present case showed the unusual findings of parotid involvement, possible pericardial involvement and, microscopically, early neutrophilic perivascular infiltration.

Encouraging results with epinephrine therapy were noted, and the possibility of more dramatic therapeutic response with the 11,17-oxysteroids proven to be of value in other diffuse collagen diseases has been discussed.

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Book Reviews

Psychosomatic Medicine. By Franz Alexander, M.D. Cloth binding, 300 pages. W. W. Norton & Co., Inc. New York, 1950. *Price* $4.00.

Dr. Alexander has stressed the importance of the psychodynamic approach to various syndromes that at one time were exclusively in the field of organic medicine. In the first part of his book he has defined psychosomatic medicine and given the basic principles. The psychosomatic diseases and the conversion hysterias have been clearly differentiated. Dr. Alexander has given in general the unconscious mechanisms which cause the various psychosomatic disorders. He is not at all impressed with any common personality or character trait which would tend toward any particular psychosomatic disease.

In the second part of his book the most common psychosomatic disorders have been considered individually with their unconscious mechanism. For example, in the gastrointestinal diseases he has considered repressed dependencies as the motivating conflict, while in the cardiovascular disorders the repressed hostilities, are the motivating factors. There are plenty of examples and case histories which make his book very easy reading.

His chapter on therapy gives an encouraging outlook in the therapy of these conditions. Psychoanalysis is the method employed but psychotherapy is also beneficial.

The book has recognized the tremendous importance of psychosomatic medicine and Dr. Alexander clearly recognizes the tremendous growth in store for it. It is a book that everyone could read with definite benefit.

S.M.D.

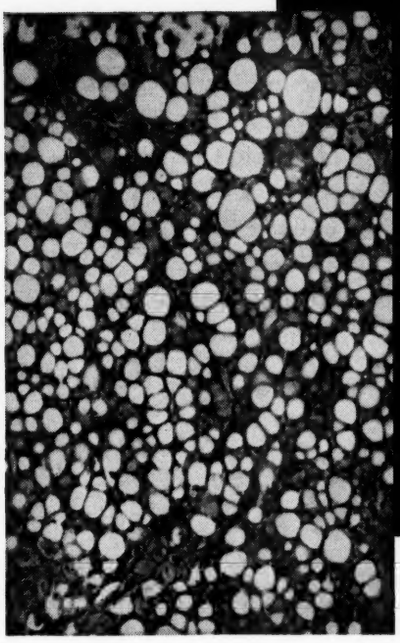
A B C's of Sulfonamide and Antibiotic Therapy. By Perrin H. Long, M.D., 231 pages. Philadelphia. W. B. Saunders Co.

The objective of the author to present a concise, up-to-date, authoritative guide to use of the various sulfonamide and antibiotic drugs has been admirably achieved. The book covers the various means of administration of the different agents, their toxic reactions and a brief discussion of the accepted therapeutic approach to the various infectious diseases. To facilitate the use of the book these diseases have been arranged alphabetically. No references are included.

A.R.L.

CHOLINE LIPOTROPIC ACTION

IN THE PREVENTION AND REVERSAL OF FATTY INFILTRATION



PATIENT . . . Middle aged female, with history and findings suggesting cirrhosis: loss of appetite, nausea, vomiting, vague gastrointestinal complaints, enlarged liver. Liver biopsy showed extensive fatty changes without fibrosis, indicating that the condition would be still amenable to treatment.

REGIMEN . . . High protein, high carbohydrate, moderate fat, reinforced with vitamin therapy and the lipotropic agent, *Choline (Flint)*. Patient remained ambulatory, except for short period of hospitalization required for biopsy.

RESULTS . . . At the end of four weeks' treatment, a second biopsy was taken, revealing an entire disappearance of the fatty changes. All signs and symptoms of hepatic failure had disappeared.

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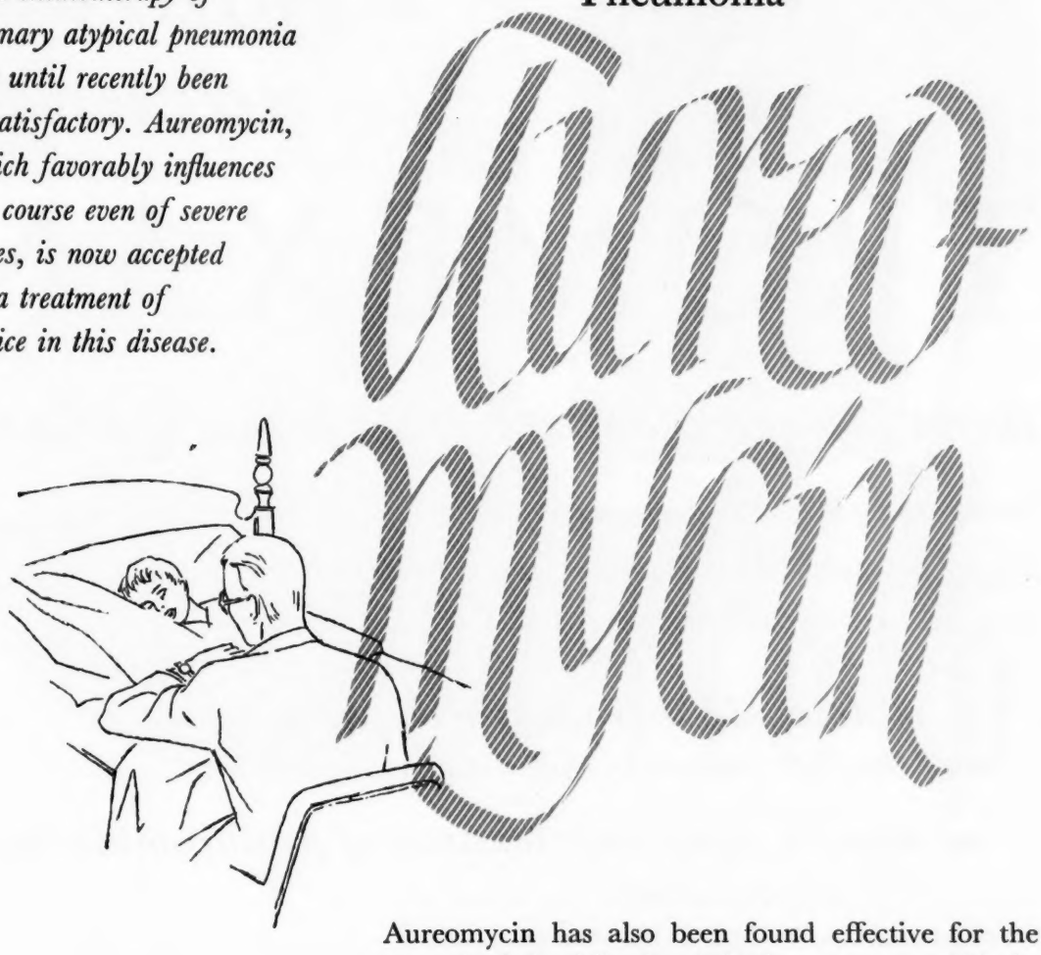
in Primary Atypical Pneumonia

The chemotherapy of primary atypical pneumonia has until recently been unsatisfactory. Aureomycin, which favorably influences the course even of severe cases, is now accepted as a treatment of choice in this disease.

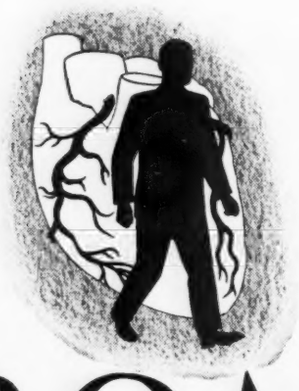


Capsules: Bottles of 25, 50 mg. each capsule. Bottles of 16, 250 mg. each capsule.

Ophthalmic: Vials of 25 mg. with dropper; solution prepared by adding 5 cc. of distilled water.

Aureomycin has also been found effective for the control of the following infections: acute amebiasis, bacterial and virus-like infections of the eye, bacteroides septicemia, boutonneuse fever, acute brucellosis, common infections of the uterus and adnexa, resistant gonorrhea, Gram-positive infections (including those caused by streptococci, staphylococci, and pneumococci), Gram-negative infections (including those caused by the coli-aerogenes group), granuloma inguinale, *H. influenzae* infections, lymphogranuloma venereum, psittacosis (parrot fever), Q fever, rickettsialpox, Rocky Mountain spotted fever, subacute bacterial endocarditis resistant to penicillin, surgical infections, tick-bite fever (African), tularemia and typhus.

LEDERLE LABORATORIES DIVISION AMERICAN Cyanamid COMPANY 30 Rockefeller Plaza, New York 20, N. Y.



a most significant advance

TROMEXAN T. M.

ethyl acetate

new, safer, oral anticoagulant

Throughout the exhaustive studies on TROMEXAN, involving many hundreds of cases, this new anticoagulant has proved singularly free from the dangers of hemorrhagic complication. Other advantageous clinical features of TROMEXAN are:

1 ***more rapid therapeutic response***
(therapeutic prothrombin level in 18-24 hours);

2 ***smooth, even maintenance of prothrombin level***
within therapeutic limits;

3 ***more rapid return to normal***
(24-48 hours) after cessation of administration.

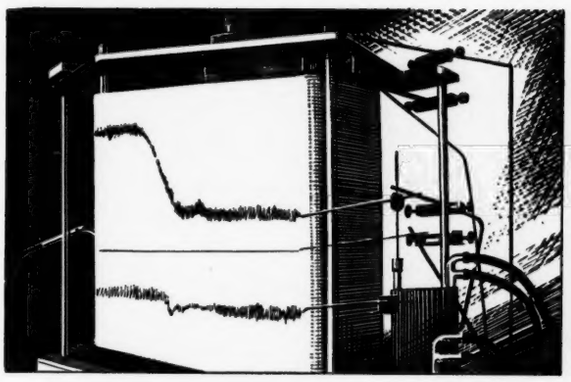
In medical and surgical practice . . . as a prophylactic as well as a therapeutic agent . . . TROMEXAN extends the scope of anticoagulant treatment by reducing its hazards.

Detailed Brochure Sent on Request.

TROMEXAN (brand of ethyl biscoumacetate): available as uncoated scored tablets, 300 mg., bottles of 50 and 250.

Geigy

GEIGY COMPANY, INC.
Pharmaceutical Division, 89-91 Barclay St., New York 8, N. Y.



VERILOID*

Product of Riker Research

In every form of hypertension, a new and more efficacious approach

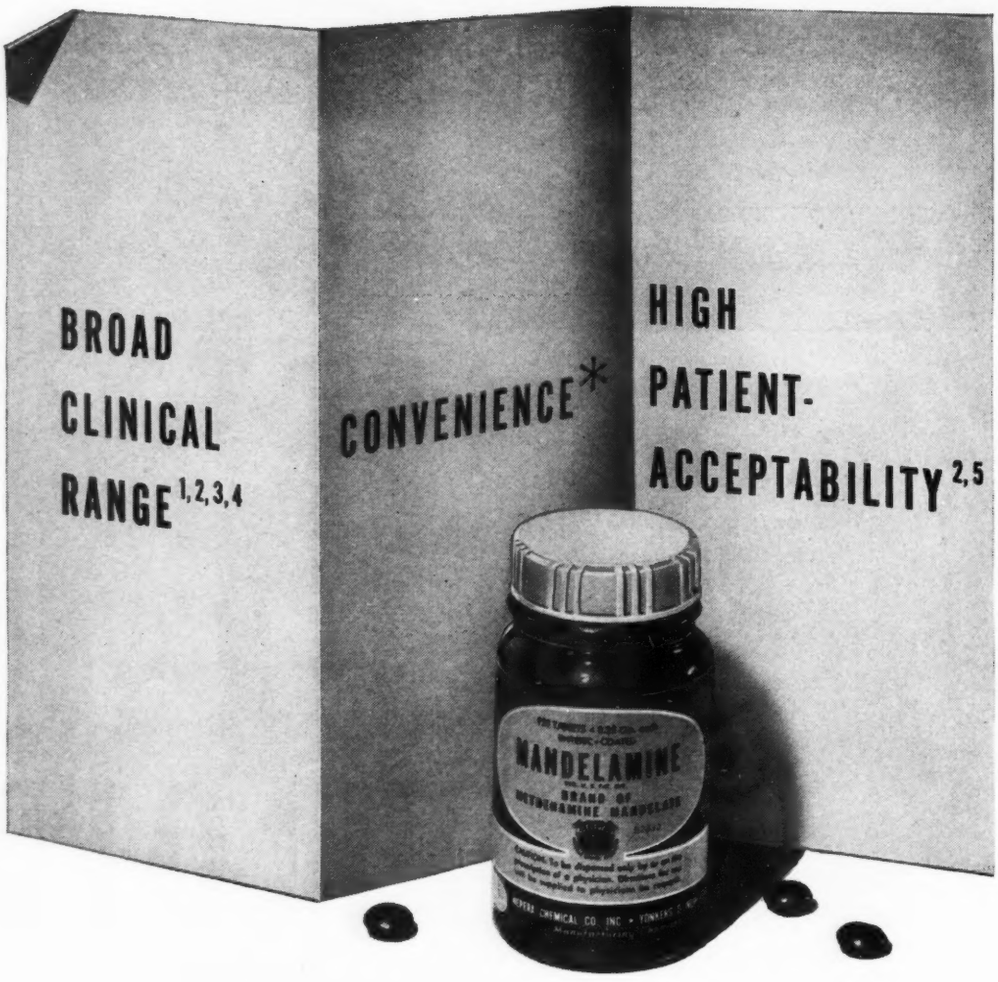
UNIFORM POTENCY. Careful biologic standardization of Veriloid in dogs, *using drop in blood pressure as the end point,* insures uniformity in hypotensive potency. Veriloid makes available for the first time the active ester alkaloids of Veratrum viride in rigidly assayed form, making for greater accuracy in the management of the hypertensive patient.

UNIFORM THERAPEUTIC EFFECT. Veriloid is uniform in its therapeutic effect—from bottle to bottle, from batch to batch. While individualization of dosage is essential for maximum therapeutic benefit, in the majority of patients the average dose of 2.0 to 5.0 mg. three or four times daily, after meals and at bedtime, leads to prompt objective and subjective improvement. Adjustment of dosage thereafter to suit the specific needs of the patient is accomplished in two to three weeks. Therapy can then be maintained as long as necessary since tolerance to Veriloid is not likely to develop.

Veriloid is available on prescription only through all pharmacies in tablets containing 1.0 mg., in bottles of 100, 200, 500, and 1000. Literature on request.

*Trade Mark Riker Laboratories, Inc.

RIKER LABORATORIES, INC., 8480 BEVERLY BLVD., LOS ANGELES 48, CALIF.



Specify MANDELAMINE® *in Urinary Antisepsis*

[BRAND OF METHENAMINE MANDELATE]

to establish and maintain urinary antisepsis in the management of pyelitis, pyelonephritis, nephroptosis with pyelitis, cystitis, prostatitis, nonspecific urethritis, and infections associated with urinary calculi or neurogenic bladder; and for pre- and postoperative prophylaxis in urologic surgery.

SUPPLIED: Bottles containing 120, 500, and 1,000 enteric-coated tablets; each tablet 0.25 Gm.

1. Scudi, J. V., and Reinhard, J. F.: J. Lab. & Clin. Med. 33: 1304 (1948). 2. Carroll, G., and Allen, N. H.: J. Urol. 55: 674 (1946). 3. Simons, I.: J. Urol. 62: 595 (1949). 4. Butt, A. J.: J. Florida M. A. 35: 430 (1949). 5. Merricks, J. W.: West Virginia M. J.: 44: 157 (1948).

ACCEPTED COUNCIL ON PHARMACY AND CHEMISTRY AMERICAN MEDICAL ASSOCIATION

6 OUTSTANDING FEATURES

1 Wide antibacterial range — including both gram-negative and gram-positive organisms
2 No supplementary acidification required (except when urea-splitting organisms occur)
3 Little or no danger of drug-fastness
4 Exceptionally well tolerated
5 No dietary or fluid regulation
*6 Simplicity of regimen—3 or 4 tablets t. i. d.

Literature and Samples on Request

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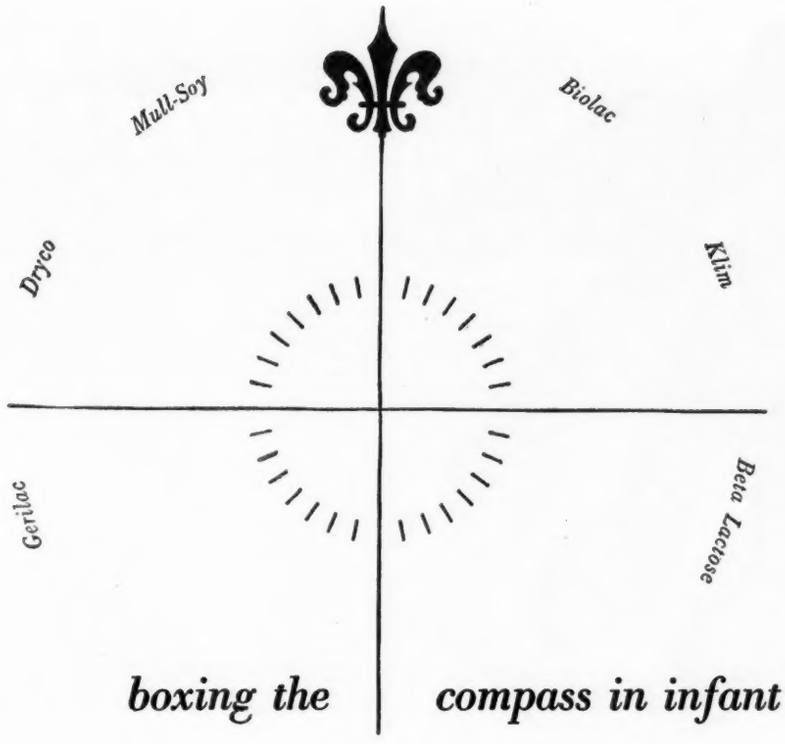
NEPERA CHEMICAL CO., INC. *Pharmaceutical Manufacturers* **NEPERA PARK · YONKERS 2, N. Y.**



boxing the compass in infant nutrition

North, East, South, West—for every type of nutritional requirement, there is a Borden prescription product scientifically designed to meet the problem.

BIOLAC, Borden's improved, evaporated-type liquid modified milk, provides for all the known nutritional needs of early infancy except vitamin C.

DRYCO, a high-protein, low-fat powdered milk, serves as a valuable food in itself and as a versatile base assuring ample protein intake plus vitamins A and D. MULL-SOY is the answer to milk allergies—an emulsified hypo-allergenic soy food approximating milk. GERILAC, a spray-dried whole milk and skim milk powder, supplies elderly patients with high quality protein, calcium and iron, and also vitamins A, D, B and C. BETA LACTOSE promotes normal intestinal flora and acidity when used as a carbohydrate modifier. KLIM is powdered pasteurized whole milk, spray-dried for rapid solubility, convenient in hot climates and during travel.

These Borden products conform to the requirements of the Council on Foods and Nutrition and the Advertising Committee of the American Medical Association and are available only in pharmacies. We welcome inquiries from physicians. Write for professional literature and attractive practical Recipe Books.

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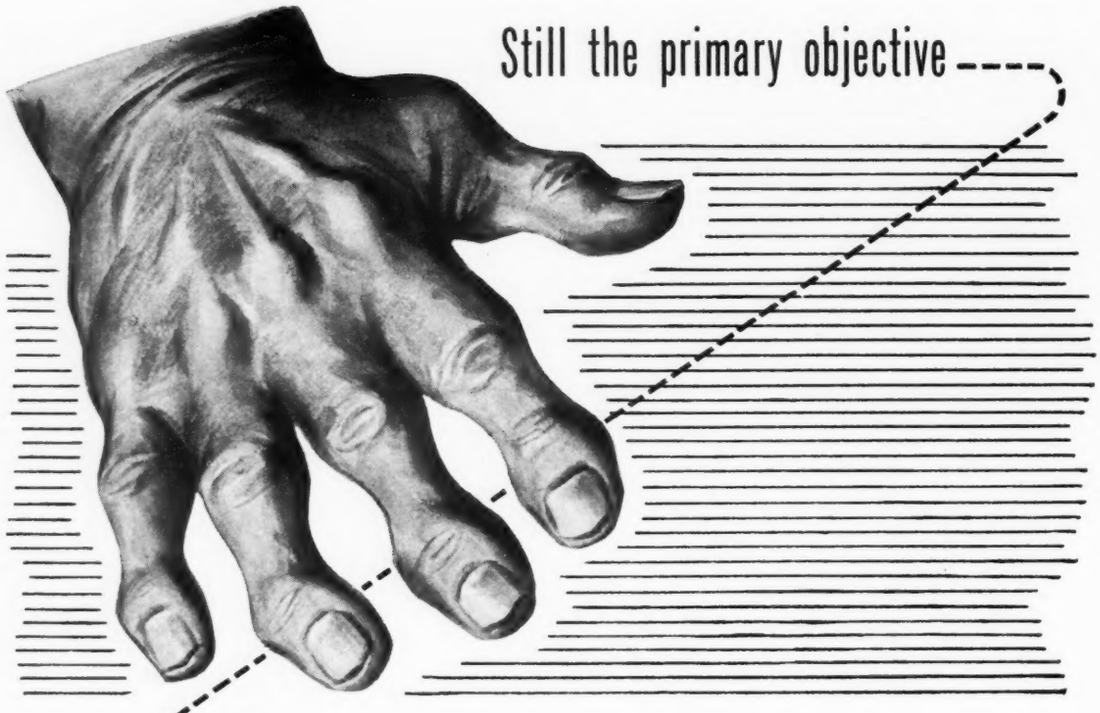


Still the primary objective ----

Relief of arthritic pain

With Salcedrox, relief of arthritic pain is more positive than from salicylates alone.

Salcedrox may be given in adequate dosage to produce the high blood levels needed in the treatment of arthritis, rheumatic fever, neuritis, and allied conditions.

Because of the buffering agents present, Salcedrox is readily tolerated by the gastric mucosa. Its content of calcium reduces the systemic toxicity of sodium salicylate and the calcium ascorbate corrects the vitamin C deficiency frequently seen in rheumatic states.

Available on prescription through all pharmacies. Literature available on request.

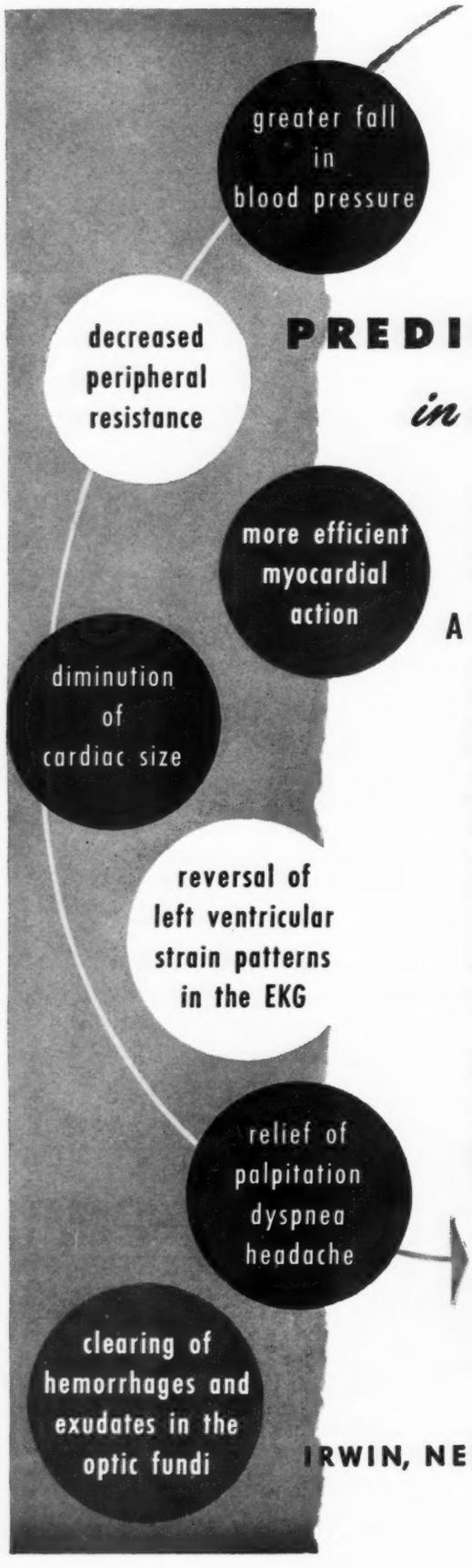
THE S. E. MASSENGILL COMPANY
Bristol, Tenn.-Va.
NEW YORK • SAN FRANCISCO • KANSAS CITY

SEMCO

Each Salcedrox Tablet supplies:

Sodium salicylate	5 gr.
Aluminum hydroxide gel, dried	2 gr.
Calcium ascorbate (equivalent to 50 mg. ascorbic acid)	1 gr.
Calcium carbonate	1 gr.

Salcedrox
TABLETS



PREDICTING RESPONSE

in essential hypertension

Striking objective improvement, in the individual case, can be obtained with veratrum viride Biologically Standardized in Craw Units, as available in VERATRITE and VERTAVIS.

A Therapeutic Alternative

In mild and moderate hypertension (Grades I and II), which accounts for more than 70 per cent of all hypertensive cases, VERATRITE is the choice of therapy and may be used routinely in everyday practice without undesirable side-effects. VERATRITE contains, in each tabule, veratrum viride (3 Craw Units) with sodium nitrite and phenobarbital.

In severe, resistant hypertension (Grade III) *and hypertension complicated by cardiac failure,* VERTAVIS can effect dramatic response. Adequate supervision of the patient and fine adjustment of dosage to the individual case are essential. VERTAVIS is a single agent, containing in each tablet veratrum viride (10 Craw Units).

Samples and literature on both VERATRITE and VERTAVIS, including clinical reports, are available on request.

Veratrite®
for mild and moderate hypertension

Vertavis®
for severe, resistant hypertension

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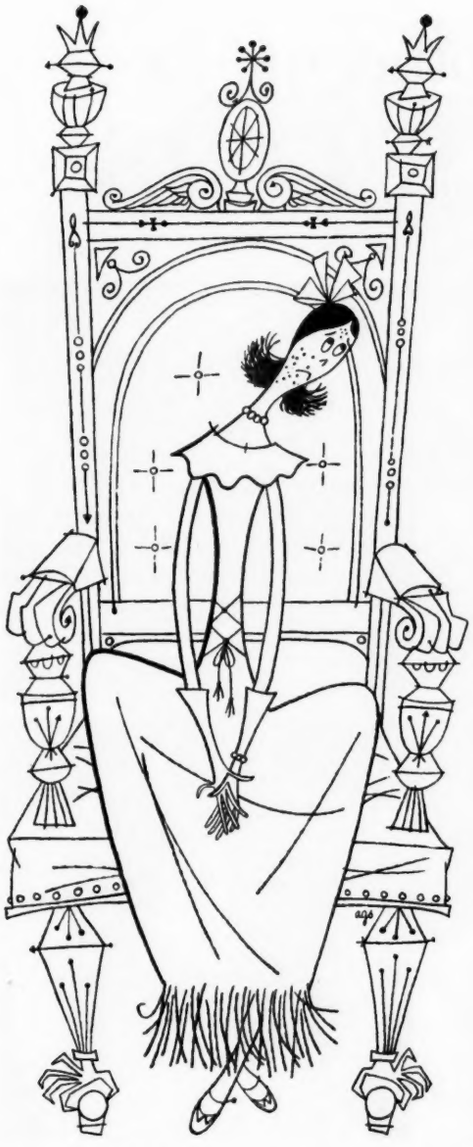

to help

your acne patient

get back "in the swing"

Your complexion-conscious young acne patient regains her self-confidence when you write 'ACNOMEL'. Because ACNOMEL is:

1 ***Immediately effective cosmetically.*** **ACNOMEL is delicately flesh-tinted. It masks the acne lesions, yet is virtually invisible when applied.**

2 ***Rapidly effective therapeutically.*** **ACNOMEL ordinarily brings definite improvement—not in months or weeks, but in a matter of days.**

Formula: Resorcinol, 2%; and sulfur, 8%; in a stable, grease-free, flesh-tinted vehicle.

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Acnomel

'Acnomel' T.M. Reg. U.S. Pat. Off.

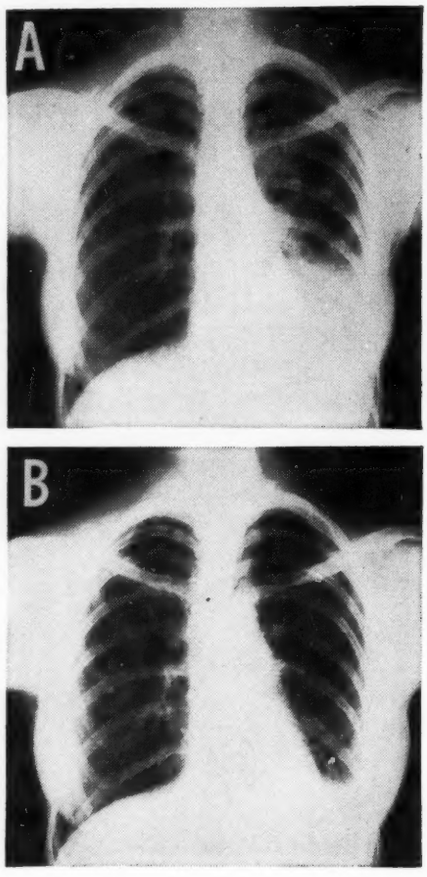
a significant advance, clinical and cosmetic, in acne therapy

Effective Chemotherapy in TUBERCULOSIS

DIHYDROSTREPTOMYCIN or Streptomycin, used alone or in combination with para-aminosalicylic acid, is recognized as a valuable and, in some instances, an essential adjuvant in the treatment of selected types and stages of tuberculosis.

Para-aminosalicylic acid is capable of inhibiting or significantly delaying the emergence of bacterial resistance to dihydrostreptomycin or streptomycin.

These drugs are not to be regarded as substitutes for traditional therapeutic methods. Rather, they serve best when properly integrated with bed rest and, where necessary, collapse measures or other forms of surgery.



A. Before Treatment *(9 days prior to Dihydrostreptomycin therapy) Diffuse lobular tuberculous pneumonia, lower half of left lung; thin-walled cavity above hilus (3 x 3.5 cm.).*

B. After 3 Months' Treatment *(2 days after discontinuance of Dihydrostreptomycin) Considerable clearing of acute exudative process in the diseased lung; cavity smaller and wall thinner.*

Detailed literature on the subject of chemotherapy in tuberculosis will be supplied upon request.

COUNCIL ACCEPTED

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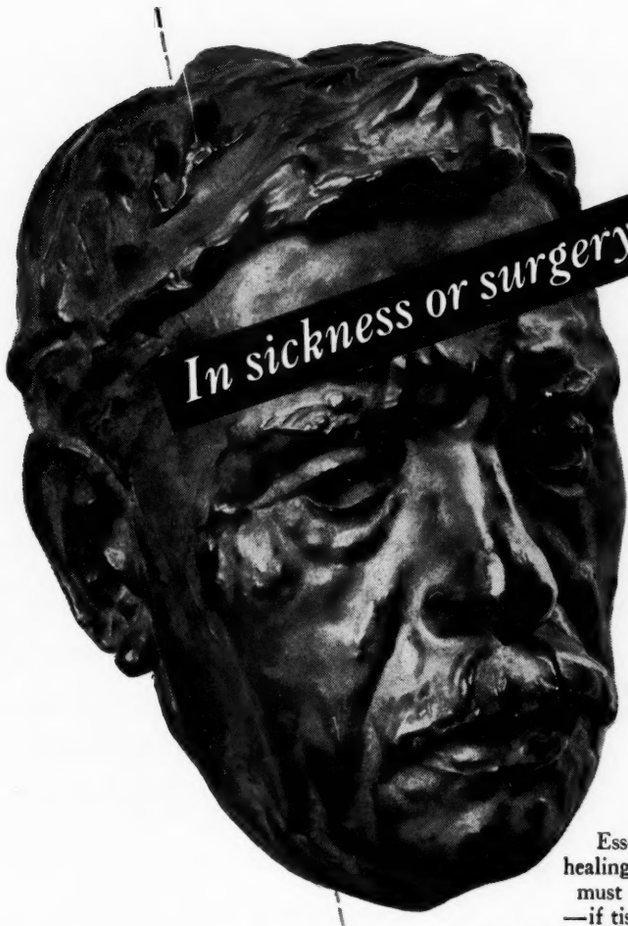
Manufacturing Chemists
RAHWAY, NEW JERSEY

Merck Antituberculosis Agents

Streptomycin Calcium Chloride Complex Merck

Para-Aminosalicylic Acid Merck (PAS)

Crystalline Dihydrostreptomycin Sulfate Merck



In sickness or surgery, play safer with . . .

"Saturation Dosage"

of vitamins b *and* c

Depletion of the critical water-soluble B complex and C vitamins occurs so commonly in the presence of physical pathology, as to make a presumption of nutritive impairment[2] almost axiomatic. Essential to normal cell metabolism and wound healing, these poorly-stored, readily-diffusible factors must be replenished — usually by *massive dosage* —if tissue rehabilitation[3] and return to health[4] are to be expedited. • Allbee with C 'Robins' provides this all-important "saturation dosage" in convenient capsule form. It incorporates the important B factors in 2 to 15 times daily requirements, plus 250 mg. of vitamin C — *the highest strength of ascorbic acid available today in a multi-vitamin capsule.* • Its prescription represents a sound contribution toward decisive recovery from disease, or toward pre- and post-operative nutritional support.[1]

A. H. ROBINS CO., INC. • RICHMOND 20, VA.
Ethical Pharmaceuticals of Merit since 1878

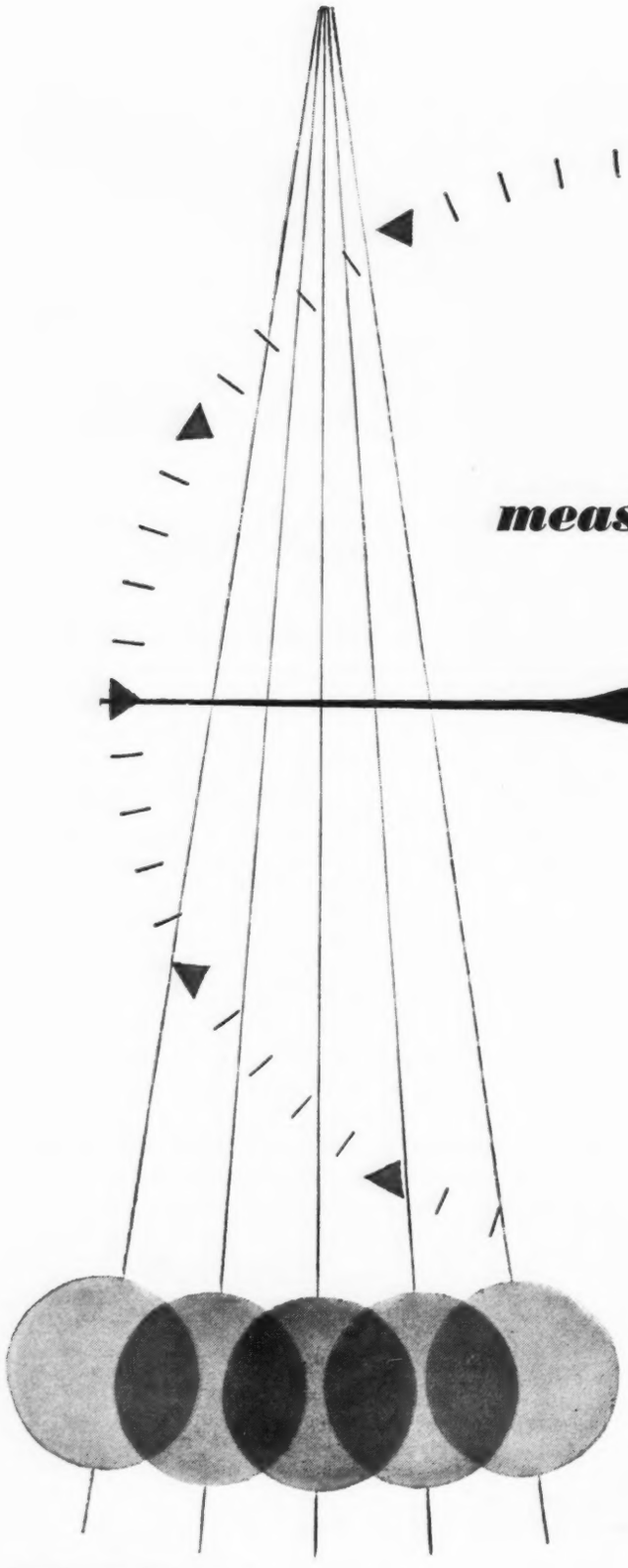
FORMULA: ***Each Allbee with C capsule contains:***

Thiamine hydrochloride (B_1)	15 mg.
Riboflavin (B_2)	10 mg.
Nicotinamide	50 mg.
Calcium pantothenate	10 mg.
Ascorbic acid (C)	250 mg.

REFERENCES: 1. Coller, F. A. and DeWeese, M. S.: Preoperative and Postoperative Care, J.A.M.A., 141:641, 1949. 2. Jolliffe, N. and Smith, J. J.: Med. Clin. North America, 27:567, 1943. 3. Kruse, H. D.: Proc. Conf. Convalescent Care, New York Acad. Med., 1940.
4. Spies, T. D.: Med. Clin. North America, 27:273, 1943.

allbee® WITH C

Robins



measured in minutes

Rapid anticoagulant effects are available with Heparin Sodium preparations, developed by Upjohn research workers. In a matter of minutes, coagulation time can be lengthened to offset danger from thrombosis and embolism. With Depo*-Heparin Sodium, prolonged effects lasting 20 to 24 hours may be obtained with a single injection. Therapy with these Upjohn anticoagulants is distinguished by promptness of action, simplicity of supervision, and ready controllability.

**Trademark, Reg. U. S. Pat. Off.*

Upjohn ***Medicine...Produced with care...Designed for health***

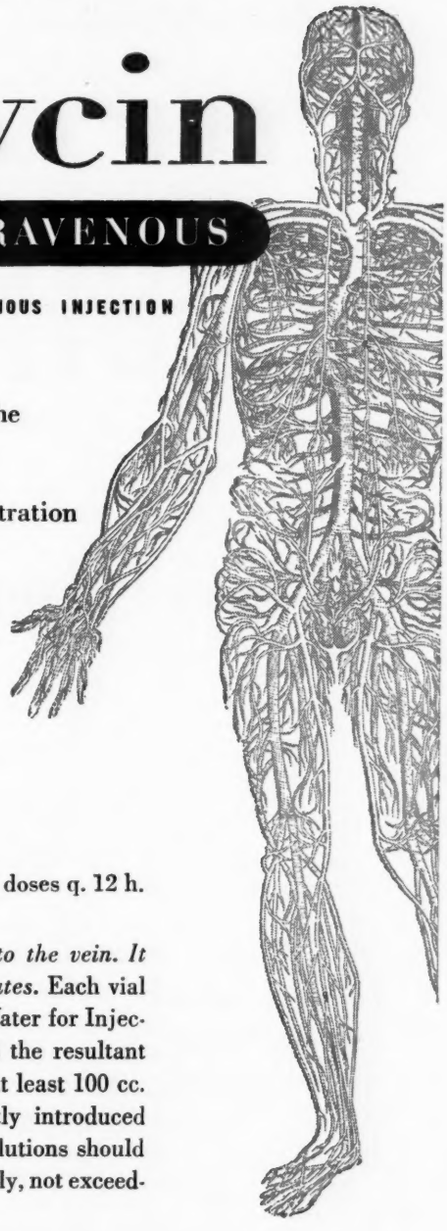
THE UPJOHN COMPANY, KALAMAZOO 99, MICHIGAN

Pfizer announces...

Terramycin

for hospital use only **INTRAVENOUS**

CRYSTALLINE TERRAMYCIN HYDROCHLORIDE FOR INTRAVENOUS INJECTION



Affords the advantages of intravenous therapy with the newest of the broad-spectrum antibiotics in:

... those conditions and cases in which oral administration is not feasible;

... severe, fulminating or necrotizing infections (by rapidly producing high serum concentrations);

... pre- and post-operative prophylaxis;

... peritonitis.

Dosage and Administration:

0.5 Gm. to 1.0 Gm. of Terramycin Intravenous in divided doses q. 12 h. has been found adequate for most acute infections.

Terramycin Intravenous should be injected directly into the vein. It is never given by the intramuscular or subcutaneous routes. Each vial is dissolved in sterile 5% Dextrose for Injection, USP, Water for Injection, USP, or Physiological Saline Solution, USP, and the resultant clear solution further diluted to give a final volume of at least 100 cc. When desired, Terramycin Intravenous may be directly introduced into solutions for continuous drip infusion. Injection solutions should not contain more than 5 mg. per cc. and are injected slowly, not exceeding 100 cc. in five minutes.

Supplied: 10 cc. vials containing 250 mg. of Crystalline Terramycin Hydrochloride with sodium glycinate as a buffer.
20 cc. vials containing 500 mg. of Crystalline Terramycin Hydrochloride with sodium glycinate as a buffer.

Antibiotic Division **Pfizer** CHAS. PFIZER & CO., INC., *Brooklyn 6, N.Y.*

*Intermediate Acting**

GLOBIN INSULIN

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**Clinical Evidence:—*

"...it was found that the characteristic activity of globin insulin and 2:1 mixture (of protamine zinc and regular) insulin is essentially the same."[1]

"Not often do either globin insulin or a 2:1 mixture require supplementary use of regular insulin. Fully 80% of all severe diabetics can be balanced satisfactorily with one of them."[2]

1. *Reeb, B. B., Rohr, J. R., and Colwell, A. R.: Proc. House Staff Dept. Med., Wesley Memorial Hospital, Chicago, Ill. Feb. 6, 1948.*

2. *Rohr, J. H., and Colwell, A. R., Proc. Amer. Diabetes Assn. 8:37, 1948.*

COMPLETE CLINICAL INFORMATION WILL BE SENT ON REQUEST

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